LONGITUDINAL EVALUATION OF SELF-REPORTED ANTIBIOTIC USE AND ITS ASSOCIATION WITH VAGINAL AND RECTAL COLONIZATION BY LACTOBACILLUS AND VULVOVAGINAL CANDIDIASIS IN NON-PREGNANT WOMEN

by

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ABSTRACT

This dissertation includes three manuscripts describing the use of systemic and intra-vaginal antibiotics in non-pregnant women. The first manuscript described the scope of antibiotic use and showed that the antibiotic use rate was high. Nearly half of the antibiotics were used to treat genitourinary infections. However, one in five antibiotics were used to treat upper respiratory tract illnesses for which antibiotics are not indicated according to CDC recommendations and these antibiotics were primarily β-lactam agents.

The second manuscript evaluated whether antibiotic use impacted vaginal and rectal colonization by hydrogen peroxide (H_2O_2)-producing lactobacilli, which are the predominant members of the healthy vaginal microbiome. These analyses showed there was a significant reduction in vaginal colonization by H_2O_2-producing lactobacilli following the use of β-lactam antibiotics, while other classes of antibiotics had no measurable effect on *Lactobacillus* colonization. A novel finding was that β-lactam use also reduced rectal colonization by lactobacilli to a similar degree as that observed for the vagina and that the effects persisted for a longer period of time.

The third manuscript evaluated whether antibiotic use was associated with acquisition of vaginal yeast infections. Antibiotic use was associated with increased acquisition of yeast vaginitis, and while the highest risk was associated with the use of β-lactam antibiotics, the use of some other classes of antibiotics, including metronidazole, fluoroquinolones, and nitrofurantoin, were also associated with increased yeast vaginitis.

Each manuscript provided new insights into the public health significance of antibiotic use in reproductive-aged women. The first confirmed that antibiotic use was common in women and extended our knowledge by showing that the treatment of genitourinary tract infections was the primary indication for antibiotic use and that treatment of upper respiratory tract infections was the major contributor to exposure of women to β-lactam antibiotics. The results of the second and third manuscripts suggest that decreasing the inappropriate use of β-lactam antibiotics deserve special attention since β-lactams were associated with decreased colonization by beneficial lactobacilli and increased yeast vaginitis. This research has provided insights on how efforts to reduce antibiotic use should be tailored in young women for the greatest public health benefit.

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1.0 INTRODUCTION

In the early 1900's, the leading cause of mortality in the United States was infectious diseases. From 1938 to 1952, there was a rapid decline in infectious disease mortality rates which was partially attributable to the introduction of antimicrobials in clinical care; sulfonamides in 1935 and antibiotics in 1941.[1, 2] While antibiotics have been beneficial in treating infections due to pathogenic bacteria, inappropriate antibiotic use in humans and in commercial agriculture has contributed to a new public health threat, the emergence of antibiotic resistant bacteria. The Centers for Disease Control and Prevention (CDC) estimate that antibiotic resistant infections cause more than 2 million illnesses and 23,000 deaths per year in the United States.[3] Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common antibiotic-resistant pathogen identified in hospitals and accounted for 278,000 hospitalizations in the United States in 2005.[4] The proportion of *S. aureus* isolates from intensive care unit patients that were MRSA nearly doubled between 1992 and 2003 from 36% to 64%, respectively.[5] Similar trends have been identified for other important pathogens such as *E. coli*, the most common cause of bloodstream infections, *Streptococcus pneumoniae*, a common cause of respiratory tract infections[6, 7], and *Neisseria gonorrhoeae*, a sexually transmitted infection.[8] To address this problem, the CDC launched a national campaign in 1995 to promote appropriate antibiotic use.[9] However, in spite of the recognized impact of inappropriate antibiotic use, antibiotics were prescribed in 10% of all ambulatory visits among adults from 2007 to 2009, and during 46% of visits for respiratory conditions where antibiotics are rarely indicated.[10] These data suggest that inappropriate antibiotic use is still prevalent.

In addition to contributing to an increase in multidrug resistant pathogens, antibiotic use can also have a deleterious impact on commensal bacteria which colonize the mucosal and skin surfaces of humans, such as the gastrointestinal, respiratory and reproductive tracts. The critical role of the human microbiome in human health has been increasingly recognized.[11] Disturbance of the microbiome following antibiotic use can allow the overgrowth of pathogens which can cause life-threatening infections. The most well characterized example of this is the disturbance of the normal intestinal microflora that leads to antibiotic-associated diarrhea and the increased incidence of pseudomembranous colitis associated with *Clostridium difficile* following antibiotic therapy.[12, 13] Fecal transplants are a safe and effective mechanism to replenish the gut microflora as an adjunctive treatment for this syndrome.[13] The detrimental impact of antibiotics on beneficial vaginal bacterial such as lactobacilli has also been documented.[14] However, few studies have evaluated the effect of antibiotic use on the commensal bacteria of the vagina and rectum.

Lactobacillus species are the predominant bacteria in the vagina, and *Lactobacillus crispatus* have been shown to be an important predictor of a stable vaginal microbiome.[15] Lactobacilli produce lactic acid which maintains an acidic environment in the vagina, and some species produce hydrogen peroxide which is toxic to other organisms. These metabolic products are thought to protect the vagina from colonization by pathogenic bacteria. Women who harbor hydrogen peroxide–producing lactobacilli in the vagina and/or the rectum have a reduced incidence of bacterial vaginosis, which is the most common vaginal syndrome among reproductive-age women.[16, 17] Bacterial vaginosis is characterized by the absence of lactobacilli and the overgrowth of *Gardnerella vaginalis* and a diverse group of anaerobic bacteria, and is associated with increased acquisition of sexually transmitted infections including human

immunodeficiency virus (HIV), herpes simplex virus type 2, *N. gonorrhoeae*, *Chlamydia trachomatis*, and *Trichomonas vaginalis*.[18-22] There have been few studies which evaluated the effect of antimicrobial use on vaginal colonization by *Lactobacillus* beyond those used for the treatment of bacterial vaginosis.[23, 24] Many of the published studies are small and have conflicting results.[14, 25-32] Therefore, it is unclear whether antibiotics being used for other indications in women are having a deleterious impact on vaginal lactobacilli and thereby increasing their risk for acquiring vaginal infections.

Vulvovaginal candidiasis (VVC) is a common cause of vaginitis symptoms among women of reproductive age and is characterized by vulvar itching. It is estimated that as many as three out of four women will have VVC at least once in their lifetime.[33] The prevalence of yeast colonization, primarily by *Candida* species, is 20-25% among women of childbearing age with vaginal symptoms, while 10% of women are asymptomatically colonized by yeast in the vagina.[34, 35] Among women reporting an initial yeast infection, 10-25% reported four or more recurrent infections per year.[36] Based on 1995 estimates, the annual cost of clinical visits and treatment for VVC is projected to be $3.1 billion in 2014.[37] In addition to the discomfort and costs associated with VVC, some prospective studies have shown an increased risk of HIV acquisition among women who had VVC at the visit prior to sero-conversion.[38, 39] While the pathogenesis of VVC remains unclear, several risk factors have been identified including systemic antimicrobial use and absence of vaginal *Lactobacillus* colonization.[33] However, the results regarding antimicrobial use as a risk factor for incident vulvovaginal candidiasis have been inconsistent across studies.[40-48]

In 2010, there were 801 antibiotic prescriptions per 1000 persons in the United States.[49] While antibiotic prescribing patterns have been frequently described in outpatient and inpatient

hospital populations, children, and the elderly, there are few reports about antibiotic use specifically in women.[50-55] A study of antibiotic prescribing patterns in general practices in the United Kingdom, reported that 39% of all females, including children, were prescribed an antibiotic in 1996 for an overall rate of 852 per 1000 patient years which was higher than the rate in males (607 per 1000 patient years).[56] Two European studies that compared antibiotic use during pregnancy with the year prior to pregnancy found that antibiotic prescribing was higher during the non-pregnant period.[57, 58] Studies on antibiotic prescribing patterns in non-pregnant women are needed in order to understand the burden of antibiotic use in this population and their potential for adversely affecting the indigenous vaginal microflora and subsequently increasing the risk for acquiring vaginal infections such as VVC, bacterial vaginosis, and HIV.

The objective of the present research is to describe the frequency of and reason for antimicrobial use in healthy, non-pregnant women, identify demographic and behavioral factors associated with antibiotic use and to characterize the impact of antibiotic use on vaginal and rectal colonization by *Lactobacillus spp*. and on developing vulvovaginal candidiasis. These analyses will provide novel insights into the impact of antibiotic use on colonization by commensal lactobacilli associated with health, and the impact of antibiotic use on yeast vaginitis. The following review is an overview of the published literature describing the burden of antibiotic use in the United States, the *in vitro* susceptibility of lactobacilli to classes of antibiotics, and the effects of antibiotic use for any indication on colonization of the vagina and rectum by lactobacilli and its association with vulvovaginal candidiasis.

2.0 LITERATURE REVIEW

2.1 ANTIBIOTIC CONSUMPTION

2.1.1 Regional variations in antibiotic consumption

Antibiotics are one of the most commonly prescribed classes of medications. Antimicrobial use varies by country, regions within countries, and classes of antibiotics. A study that compared the outpatient antibiotic consumption in the United States to that of 27 European countries in 2004 reported that the US ranked fourth behind Greece, France, and Italy. The antibiotic use data was based on reimbursement, distribution, or sales data. The defined daily dose per 1000 inhabitants per day (DID) for Greece, France, Italy, and the US was 33.38, 27.09, 25.69, and 24.92, respectively, while the Netherlands had the lowest antibiotic consumption at 10.00 DID. The overall consumption in the 27 European countries included in this study was 19.04 DID. Since the amount of antibiotic use was based on different data sources in each country, there may be issues with the estimates. Antibiotic consumption in Greece may have been overestimated since sales data was used which includes antibiotics that were exported to other countries. Conversely, antibiotic use may have been underestimated in the US since it was based on retail data and did not include antibiotics dispensed from government and integrated healthcare pharmacies although the authors believe the latter had little impact on the antibiotic use estimates.[52]

While the US and Europe have recently improved methods to monitor antibiotic use in response to the increasing public health threat of antibiotic resistant microorganisms, antibiotic consumption surveillance is limited in resource-limited countries. Sales of carbapenems, which

are expensive, are 2 to 3 times higher in India, Pakistan, and Egypt than in the US.[59] A study of 25 hospitals in the Mediterranean region which included the countries of Cyprus, Egypt, Jordan, Lebanon, Malta, Tunisia and Turkey, found that the median defined daily dose (DDD) per 100 bed days was 112 during 2004-2005.[60] In contrast, the median DDD per 100 bed days was 592.0 in 2011 in an academic teaching hospital in South Africa.[61] These data suggest that there is a great deal of variation in antibiotic consumption between countries world-wide.

There are regional variations in antibiotic consumption within the US. While, the national average antibiotic prescribing rate, based on number of prescriptions filled, was 801 per 1000 persons during 2010, rates tended to be higher in the southern states and lower in the western states. The states with the highest antibiotic prescribing rates were Kentucky (1,196 per 100 persons), West Virginia (1,177 per 100 persons), Tennessee (1,159 per 100 persons), and Missouri (1,136 per 100 persons), while the states with the lowest antibiotic prescribing rates were Alaska (510 per 100 persons), Hawaii (543 per 100 persons, California (554 per 100 persons), and Oregon (556 per 100 persons). The antibiotic prescribing rate in Pennsylvania was slightly lower than the national average (787 per 1000 persons).[49]

2.1.2 Distribution of antibiotic classes consumed

While the most commonly prescribed classes of antibiotics varies by country, penicillins are the most common in the US and Europe.[49, 52] In 2010, 31% of the antibiotics dispensed in the US were penicillins, followed by macrolides (27%), cephalosporins (14%), fluoroquinolones (12%), and tetracyclines (8%).[49] While penicillins were also the most commonly used antibiotics in 27 European countries based on 2004 estimates, the relative amounts of other antibiotic classes varied between countries. In some countries such as Greece, Italy, Hungary, Denmark, and

Austria, fluoroquinolones were the second most common class of antibiotic, followed by cephalosporins, and then macrolides. Cephalospronis were more commonly used than macrolides and fluoroquinolones in Belgium, Croatia, Luxembourg, and Israel.[52]

2.1.3 Indications for antibiotic use

The conditions for which antibiotics are prescribed vary in relative frequency depending on the population served and clinical setting. In studies that have evaluated ambulatory and outpatient care settings, nursing homes, and women before and during pregnancy, antibiotics were most often used to treat upper respiratory illnesses, followed by genitourinary conditions, skin and soft tissue infections, and digestive conditions.[10, 58, 62-63] In children, the most common indication for antibiotic use is otitis media which is usually preceded by an upper respiratory infection.[54]

Upper respiratory infections are a major target in national campaigns efforts to reduce unjustified antibiotic use since studies indicate a high prevalence for over-prescribing antibiotics for these conditions. A study that evaluated an average of 985 million ambulatory visits per year among adults in the US from 2007-2009, found that antibiotics were prescribed during 101 million (10%) of these visits annually. Antibiotics were most commonly prescribed for respiratory conditions (41% of antibiotics). While antibiotics were prescribed during 65% of respiratory conditions for which antibiotics were potentially indicated, they were also prescribed during 46% of visits for respiratory conditions for which antibiotics were rarely indicated.[10] Another study that evaluated ambulatory care national survey data from 2006 to 2010 reported similar results. There were 69 million visits in which there was a diagnosis of rhinosinusitis. Antibiotics are not recommended for uncomplicated cases of acute rhinosinusitis, and confirmatory diagnostic tests are recommended before prescribing antibiotics for chronic cases.

However, antibiotics were prescribed in 85.5% of acute rhinosinusitis cases and 69.3% of chronic rhinosinusitis cases in this study.[62]

2.1.4 Antibiotic use in women

Few studies have specifically evaluated antibiotic consumption in women. A study of antibiotic prescribing patterns in general practices in the United Kingdom, reported that 39% of all females, including children, were prescribed an antibiotic in 1996 for an overall rate of 852 per 1000 patient years, while 29% of males were prescribed an antibiotic for a rate of 607 per 1000 patient years. However the antibiotic prescribing rates in reproductive-aged women (16-34 years and 35-54 years) were nearly twice as high as the rate in men in the same age groups (850 versus 473 per 1000 patient years and 739 versus 405 per 1000 patient years, respectively).[54] Two European studies that compared antibiotic use during pregnancy with the year prior to pregnancy found that overall antibiotic prescribing was slightly higher during the non-pregnant period.[57, 58] One of these studies evaluated 115,000 women who had a live birth between 1992 and 2007 in the United Kingdom. The authors reported that antibiotic prescriptions per 1000 women for respiratory and urinary tract infections in the year prior to pregnancy were 269 and 93 respectively. While the antibiotic prescribing rate during pregnancy was similar to the pre-pregnant rate for respiratory infections (228 per 1000 women), the prescribing rate for urinary tract infections nearly doubled (164 per 1000 women). The antibiotic prescribing rate without a reported indication was around 400 per 1000 women both before and during pregnancy.[58] The results of these studies indicate that antibiotic consumption is higher in reproductive-aged women than in men, and that the indications for use and subsequently the classes of antibiotics prescribed change during pregnancy.

Antibiotic stewardship programs have explored several methods to reduce unjustified antibiotic use including patient and prescriber education, clinical decision support strategies in the form of treatment algorithms, prospective audit and feedback, and formulary restrictions.[64] However, these programs have had mixed results and the world-wide prevalence of antibiotic use remains high. The use of antibiotics in commercial agriculture further increases the global antibiotic burden. The current literature is lacking in the epidemiology of antibiotic use in non-pregnant reproductive-aged women, particularly in the US which has one of the highest antibiotic consumption rates. Sexually active women are at risk for acquiring an infection by antibiotic resistant bacteria, particularly drug-resistant *N. gonorrhoeae* which is classified as an urgent public health threat in the United States.[3] Studies on antibiotic prescribing patterns in non-pregnant women are needed in order to understand the burden of antibiotic use in this population and their potential for adversely affecting the indigenous vaginal microflora and subsequently increasing the risk for acquiring vaginal infections.

2.2 ANTIBIOTIC SUSCEPTIBILITY OF VAGINAL LACTOBACILLI

2.2.1 Antibiotic susceptibility of vaginal lactobacilli *in vitro*

Lactobacilli are gram positive rods that can range from microaerophilic to strictly anaerobic. All *Lactobacillus* species produce lactic acid and most strains of some species, like *L. crispatus* and *L. jensenii*, produce hydrogen peroxide. The principal mode of action for many classes of antibiotics is the disruption of bacterial cell wall synthesis. Like most facultative gram positive bacteria, nearly all isolates of lactobacilli are susceptible to penicillins, cephalosporins, macrolides, clindamycin, and tetracycline (80-100%) and all are resistant to metronidazole and

fluoroquinolones.[65-69] There is also evidence that antibiotic susceptibility patterns vary between *Lactobacillus* species.[67-68, 70-71]

The primary species of *Lactobacillus* that colonize the vagina and rectum are *L. crispatus*, *L. jensenii*, *L. gasseri*, and *L. iners*.[72, 73] Women colonized by *L. crispatus* in the vagina have reduced incidence of bacterial vaginosis while women colonized by *L. gasseri* and/or *L. iners* are at higher risk of shifting to an abnormal microflora, which is the precursor to developing bacterial vaginosis.[15, 73-74] While most of the studies of *in vitro* antibiotic susceptibility testing have focused on the *Lactobacillus* species that are involved in food production, there are four published studies which reported results for isolates/species recovered from the genital tract.[65-68]

A Korean study evaluated the susceptibility of 108 vaginal *Lactobacillus* isolates from healthy women to 13 antimicrobial agents using the broth dilution method described by Clinical and Laboratory Standards Institute (CLSI). The majority of the isolates (76%) were identified as *L. crispatus* or *L. acidophilus*, 5% as *L. gasseri*, and 4% as *L. jensenii*. Using the CLSI interpretive breakpoints for anaerobes, all of the *Lactobacillus* isolates were susceptible to erythromycin and tetracycline, and 96% were susceptible to cefotaxime (3rd generation cephalosporin). All isolates were resistant to metronidazole. While the susceptibility of the *Lactobacillus* isolates to clindamycin was tested, the authors only reported the MIC_{90} for this antibiotic so it is not possible to determine the percentage of susceptible isolates. According to the published results, 8% of the *Lactobacillus* isolates were susceptible to penicillin and 49% were susceptible to ampicillin.[65] The unusual high rate of resistance of *Lactobacillus* isolates to penicillins in this study was unexpected and was unique to this study. It is possible that the broth dilutions were contaminated when testing these antibiotics.

A US study evaluated 36 strains of lactobacilli isolated from women who had normal vaginal flora. The *Lactobacillus* strains were not identified to the species level. The sensitivity of the lactobacilli to 11 antibiotics was performed using the agar dilution method. Only the MIC_{50}, MIC_{90}, and the range were reported so it is not possible to determine how many were susceptible to each agent. However, the authors stated that all 36 isolates were resistant to ciprofloxacin (fluoroquinolone), and that ampicillin (penicillin) and cefuroxime (2^{nd} generation cephalosporin) were highly active against lactobacilli. However, fewer than half of the isolates were sensitive to 5 other commonly used cephalosporins.[66]

A Finnish study of 85 cases of *Lactobacillus* bacteremia assessed antimicrobial susceptibility of the isolates using an agar diffusion method. The majority of clinical strains were identified as *L. rhamnosus* (n=46), *L. fermentum* (n=12), or *L. casei* (n=12). Nearly all of the clinical strains of *Lactobacillus* (95%) were susceptible to β-lactams, while susceptibility to cephalosporins varied by agent from 88% to cefuroxime to 35% to ceftriaxone. All of the isolates were susceptible to clindamycin, erythromycin, and doxycycline.[67]

Studies of clinical and probiotic *Lactobacillus* strains indicate that antimicrobial susceptibility patterns varies by species.[67-68, 70-71] A recent study evaluated the species-specific antibiotic susceptibility of 150 vaginal and rectal *Lactobacillus* strains to 5 commonly used antibiotics using the anaerobic agar dilution method. The lactobacilli were identified to the species level as follows: *L .crispatus* (n=30), *L. jensenii* (n=30), *L. gasseri* (n=30), *L. iners* (n=30), *L. vaginalis* (n=9*)*, *L. coleohominis* (n=9), *L. fermentum* (n=3), *L. ruminis* (n=3), *L. rhamnosus* (n=3), and *L. johnsonii* (n=3). While all lactobacilli tested were susceptible to cefazolin, there was species-specific heterogeneity in susceptibility to ampicillin, clindamycin, doxycycline, and azithromycin. Of 150 isolates, 125 (83%) were susceptible to ampicillin; *L.*

gasseri (100%), *L. iners* (90%), *L. vaginalis* (89%), *L. jensenii* (87%), *L. crispatus* (80%), *L. coleohominis* (67%), *L. rhamnosus* (33%), *L. ruminis* (0%), and *L. johnsonii* (0%). While most isolates were susceptible to clindamycin (80%), all *L. crispatus* and *L. jensenii*, 87% of *L. iners*, and only 13% of *L. gasseri* strains tested were susceptible to this agent. While the overall susceptibility to doxycycline was 83%, only 53% of *L. iners*, 67% of *L. jensenii* were susceptible to this antibiotic. Only 53% of *L. iners* and 67% of *L. rhamnosus* were susceptible to azithromycin while all strains belonging to other species were susceptible. The results of this study show that 80% of *L. crispatus* isolates were susceptible to ampicillin and all were susceptible to cefazolin, azithromycin, tetracycline, and clindamycin. Conversely, 90% of *L. iners* isolates were resistant to 1 or more of 4 antibiotics tested.[68]

There is variability in the *in vitro* antibiotic susceptibility patterns between *Lactobacillus* species that colonize the genital tract. *L. crispatus*, the species associated with a healthy vaginal microenvironment is highly susceptible to commonly used antibiotics while *L. iners*, the species associated with abnormal microflora is resistant.

2.2.2 Vaginal colonization by lactobacilli following antibiotic use

Of 9 published studies which evaluated the association between antibiotic exposure and vaginal *Lactobacillus* colonization, four were observational cohort studies, one was a randomized clinical trial, and four were open-label, single group trials (Table 2.1). The studies evaluated a combined total of 1496 women, although one study accounted for two-thirds of this total. Two studies evaluated self-reported antibiotic use since last visit, while the remaining seven studies evaluated the following classes of antibiotics (two studies evaluated more than one antibiotic): penicillins (n=4), macrolides (n=2), fluoroquinolones (n=2), tetracyclines (n=2), and other (n=1)

which included clindamycin and metronidazole. For the nine studies, four reported no change in colonization in lactobacilli associated with antibiotic use (two penicillins, one fluoroquinolone, and one tetracycline) while the remaining 5 reported that antibiotic use was associated with loss of lactobacilli.[14, 25-32] Only one study reported an increase in vaginal lactobacilli colonization in women who were on ampicillin therapy for the treatment of bacterial vaginosis.[26] Thus, there is little consistency in the studies, which are described in more detail below.

2.2.2.1 Vaginal colonization by lactobacilli following self-reported antibiotic use

Two prospective observational cohort studies reported that recent self-reported antibiotic use was associated with decreased vaginal *Lactobacillus* colonization. A Kenyan study of 1020 HIV-1 seronegative female sex workers evaluated the correlates of vaginal *Lactobacillus* colonization. *Lactobacillus* colonization status was assessed by culture at each bi-monthly visit and isolates were tested for hydrogen peroxide production. Women also reported during a face to face interview whether they had used antibiotics since their last visit and which type(s). Recent antibiotic use was defined as exposure to any antibiotic, excluding metronidazole, in the last 60 days. At enrollment, 22% and 10% of women were colonized by any and hydrogen peroxide-producing lactobacilli, respectively. Antibiotic use was reported at 7.2% of follow-up visits, while vaginal colonization by any lactobacilli and hydrogen peroxide-producing lactobacilli was detected at 19.0% and 8.2% of follow-up visits, respectively. Women who reported recent antibiotic use were 30% less likely to be vaginally colonized by any lactobacilli or by hydrogen peroxide-producing lactobacilli than women who denied antibiotic use, even after adjusting for demographic, behavioral, and medical factors. Additional factors that were independently associated with decreased colonization by hydrogen peroxide-producing lactobacilli included

older age, vaginal washing, *Trichomonas vaginalis* infection, and seropositivity for herpes simplex virus type 2. Women older than 40 were 50% less likely to be colonized by hydrogen peroxide-producing lactobacilli than younger women, and women who reported vaginal washing with soap and/or water were approximately 40% less likely to be colonized than those who did not. Concurrent *Trichomonas vaginalis* infection and seropositivity for herpes simplex virus type 2 were associated with a 40% and 30% decrease in hydrogen peroxide-producing *Lactobacillus* colonization, respectively. Colonization by hydrogen peroxide-producing lactobacilli was 30% more common among women with concurrent vaginal candidiasis. The strengths of this study were the large sample size and that antibiotic exposure was categorized based on antibiotic classes. The authors reported a low isolation rate of *Lactobacillus* at enrollment which may have been due to the limited culture method used in the study or the characteristics of the population. Since this study included only Kenyan commercial sex workers who engage in risky sexual behaviors and are at risk for acquiring vaginal infections, the generalizability of the results to other populations of women is limited.[14]

A smaller cohort study evaluated factors associated with sustained colonization by vaginal *Lactobacillus,* in 101 women who attended adolescent and sexually transmitted disease clinics. *Lactobacillus* colonization status was detected by culture and isolated strains were tested for hydrogen peroxide production. *Lactobacillus* cultures and self-reported antibiotic use were obtained at enrollment and at 4- and 8-months of follow-up. Of the 101 women, 96 (95%) were colonized by lactobacilli at one or more visits and 60 (59%) were colonized at all 3 visits. Of the women who were colonized at each visit, 75% to 80% were colonized by hydrogen peroxide-producing strains. Antibiotic exposure was reported at 7.2% of the nearly 8900 follow-up visits. Women who reported any antibiotic use in the last 4 months were more likely to lose

colonization by hydrogen peroxide-producing lactobacilli either by the first or second follow-up visit (38%) compared to women who did not use antibiotics (8%, $p<0.001$). The only other factor that was associated with loss of hydrogen peroxide-producing *Lactobacillus* colonization was self-reported weekly vaginal intercourse. Among 34 women who lost hydrogen peroxide-producing *Lactobacillus* colonization, 68% reported having sexual intercourse at least once a week, while only 35% of 23 women with persistent hydrogen peroxide-producing *Lactobacillus* colonization reported weekly sexual intercourse. Race, age, contraceptive methods, or number of male sexual partners were not associated with *Lactobacillus* colonization in this study. The authors did not adjust for other potential explanatory factors in their analyses and less than half of the initial study cohort of 263 women was evaluated due to loss to follow-up and out of window visits which were weaknesses of this study. Furthermore, the prevalence of antibiotic use in the cohort was not reported. The strengths of this study were the quality of the methods used to isolate and identify *Lactobacillus*.[25]

2.2.2.2 Vaginal colonization by lactobacilli following penicillin use

Four studies evaluated the effect of penicillins on vaginal *Lactobacillus* colonization. A study of 32 women, who were treated with ampicillin for 1 week, used aerobic and anaerobic culture media to detect *Lactobacillus* colonization and isolates were tested for hydrogen peroxide production. There was a 7% increase from pre-treatment levels in the prevalence of colonization by hydrogen peroxide-producing strains at 1-week, followed by a 17% increase at the 1-month visit. This increase could be due to a reduction in anaerobic bacteria since these women were being treated for bacterial vaginosis. Statistical analyses were not performed to determine whether the observed differences were significant.[26] In contrast, a randomized clinical trial

evaluated the effect of a 5-day regimen of amoxicillin on vaginal *Lactobacillus* colonization among 15 healthy, non-pregnant women, all of whom had normal *Lactobacillus* flora, as determined by a vaginal smear classification system, prior to initiation of therapy. There was a statistically significant reduction in the number of lactobacilli throughout the treatment period among 70% of the women. However, at the 1-week post-treatment visit, only 27% of women showed a reduction and all of the women, once again, had normal *Lactobacillus* flora by the 2-week post-treatment visit. A limitation of this study was that the reduction in *Lactobacillus* colonization was assessed by vaginal smear which is a semi-quantitative method based on Gram stain morphology rather than culture methods. This method is not specific to lactobacilli since other species of bacteria have similar Gram stain morphology and it does not mean that the organisms were viable.[27]

The remaining two small pilot clinical trials which included healthy women who had no exposure to antibiotics 2 to 3 months prior to enrollment, found no change in the number of vaginal lactobacilli before and after treatment with pivmecillinam for 7 days (N=20) or phenoxymethyl-penicillin for 10 days (N=6). While both these studies detected *Lactobacillus* colonization using aerobic and anaerobic cultures on selective and non-selective culture media, neither of these studies performed statistical analyses.[28,29] In addition, the strict inclusion criteria used in the phenoxymethyl-penicillin study may have been selective for women who were less likely to lose *Lactobacillus* colonization since they had to maintain normal flora throughout two menstrual cycles. This resulted in the exclusion of 8 of the 14 women initially enrolled in the study.[28]

2.2.2.3 Vaginal colonization by lactobacilli following macrolide use

Two studies evaluated the association between macrolide use and vaginal colonization by lactobacilli. A study that included 17 women treated with a single dose of azithromycin for cervicitis or a chlamydial infection, reported that while there was a 6% increase in the proportion of women colonized by any lactobacilli between the pre-treatment and 1-month post-treatment visits, the proportion of women colonized by hydrogen peroxide-producing strains decreased by 6%.[26] A smaller study evaluated the effects of a 7-day or 10-day regimen of clarithromycin on vaginal flora in 12 women being treated for sinusitis or bronchitis. In contrast to the results reported by Agnew *et al*, this study found that 4 (33%) women were colonized by lactobacilli before treatment and none were colonized at 4-6 weeks post-treatment. While it was stated that this difference was statistically significant ($P<0.025$), this result could not be duplicated using either an unpaired or more appropriate paired statistical test.[30]

2.2.2.4 Vaginal colonization by lactobacilli following fluoroquinolone use

Two studies evaluated the effect of prulifloxacin, a fluoroquinolone, on the vaginal *Lactobacillus* microflora. Tempera *et al* evaluated the effect of a 5-day regimen among 15 healthy, non-pregnant women. All of the women had normal *Lactobacillus* flora, as determined by a vaginal smear, prior to therapy and at all follow-up visits.[27] Another study that evaluated vaginal *Lactobacillus* colonization in 51 women who underwent a 14-day course of prulifloxacin therapy for symptomatic urinary tract infection found similar results. Of the 41women with baseline colonization, 38 (93%) maintained colonization at the 6-month post-treatment visit ($P=0.9$). While a vaginal swab sample for microbial evaluation was also collected at the 1- and 3-month

visits, these results were not reported and would have been more indicative of the effect of prulifloxacin on vaginal lactobacilli.[31]

2.2.2.5 Vaginal colonization by lactobacilli following tetracycline use

The association between doxycycline use and vaginal *Lactobacillus* colonization has been evaluated in two studies. A clinical trial assessed the effect of a 5-day course of doxycycline on vaginal microbes in 91 women undergoing *in vitro* fertilization. There was no difference in the proportion of women with hydrogen peroxide-producing lactobacilli before (30%) and 2-5 days after starting doxycycline therapy (31%). While it was stated that these differences were not statistically significant, the investigators did not perform a paired analysis which would have been more appropriate for these data.[32] The second study evaluated the effect of a 7-day course of doxycycline on vaginal lactobacilli in 43 women being treated for cervicitis or a chlamydial infection. While there was a 9% to 11% decrease in the proportion of women colonized by any and hydrogen peroxide-producing lactobacilli at the 1-week visit, the proportion of colonized women returned to pre-treatment levels by the 1-month visit.[26]

2.2.2.6 Vaginal colonization by lactobacilli following metronidazole and clindamycin use

The effect of intravaginal clindamycin and metronidazole therapy on vaginal *Lactobacillus* colonization was evaluated in one of the studies included in the present review. Of 73 women being treated for bacterial vaginosis, 28 used vaginal clindamycin cream for 7 days, and 45 used vaginal or oral metronidazole formulations for 5 days and 7 days, respectively. Of the women who were treated with clindamycin, 50% were colonized by any *Lactobacillus* at baseline and

29% were colonized by hydrogen peroxide-producing strains. While these proportions decreased to 25% and 11%, respectively, 1-week after initiation of therapy, there was a marked increase by the 1-month visit where 87% of women were colonized by any and 57% were colonized by hydrogen peroxide-producing *Lactobacillus*. Women who were treated with metronidazole experienced a faster restoration of normal vaginal microflora. The proportion of women colonized by any *Lactobacillus* increased from 51% at baseline to 82% by 1-week and 93% by 1-month post-treatment. While only 5 women were colonized by hydrogen peroxide-producing strains at baseline, more than half were colonized at the 1-week and 1-month visits. The large increases in vaginal *Lactobacillus* colonization observed in women who used clindamycin and metronidazole for the treatment of bacterial vaginosis was likely related to a reduction of anaerobic bacteria which enhances subsequent colonization by lactobacilli.[26]

From the results of the nine studies included in this review, there is some evidence of an association between antibiotic use and vaginal *Lactobacillus* colonization. Recent self-reported antibiotic use was associated with reduced vaginal *Lactobacillus* colonization.[14, 25] This finding was consistent in both studies in which it was evaluated and is perhaps the strongest evidence that antibiotic use may affect *Lactobacillus* colonization in the vagina since these studies accounted for 75% of the combined total of women in the studies included in this review, the studies were longitudinal so temporality between antibiotic exposure and reduction in *Lactobacillus* colonization was established, and the conclusions were verified using appropriate statistical analyses. However, both studies included women at high risk for acquiring vaginal infections and subsequently antibiotic exposure so the results may not be generalizable to other populations of women. The larger of the two studies reported a 30% reduction in vaginal *Lactobacillus* colonization following antibiotic use. This was an important finding since this

cohort, comprised of Kenyan female sex workers, had a low prevalence of vaginal *Lactobacillus* colonization (22%) at enrollment.[14] This, in addition to their occupation, puts them at high risk of acquiring vaginal infections, including HIV.[18, 19] Therefore, a reduction in vaginal colonization by lactobacilli, particularly by hydrogen peroxide-producing strains, could have a significant public health impact.

Even though penicillins and tetracyclines have an inhibitory effect on lactobacilli *in vitro*, the results of 5 studies indicated that penicillin and tetracycline use did not have any or at least a long-term effect on vaginal lactobacilli. Since these studies only included a combined total of 207 women, larger studies are needed to verify these results.[26-29, 32] Both studies which evaluated macrolides reported a slight decrease in hydrogen peroxide-producing strains of lactobacilli which was expected since macrolides inhibit gram-positive organisms *in vitro*.[26, 30] There was no association observed between vaginal *Lactobacillus* colonization and the use of fluoroquinolones which was also expected since lactobacilli are resistant to this class of antibiotics. Antibiotics used to treat bacterial vaginosis, regardless of known *in vitro* activity against lactobacilli, resulted in increased colonization by lactobacilli in the vagina.[26] These results are consistent with those reported in other studies .[23, 24]

Self-reported antibiotic use appears to impact lactobacilli which colonize the vagina. While this definition of antibiotic use included most classes of antibiotics, it is reflective of the level and variety of antibiotic exposure experienced by reproductive-aged women outside of a controlled clinical setting. The studies which prospectively assessed the effects of specific antibiotics on vaginal *Lactobacillus* colonization varied by antibiotic class and many had small sample sizes, employed vaginal smears, a suboptimal method of detecting *Lactobacillus* colonization so it is not known whether there was an effect on viable organisms, or did not

provide adequate statistical analyses to support the conclusions. There is a need for larger, better designed studies to verify the apparent lack of association between use of specific antibiotic classes and vaginal *Lactobacillus* colonization that was observed in these studies. Furthermore, this review failed to identify any studies that evaluated the association between antibiotic use and rectal *Lactobacillus* colonization. Since the rectum is considered a reservoir for many species of bacteria which colonize the vagina, it would be important to determine whether antibiotic use affects *Lactobacillus* colonization at this site.[17, 75]

Table 2.1 Summary of studies evaluating the association between vaginal *Lactobacillus* colonization and antibiotic use

Author	Study Design	Antibiotic Class: Antibiotic(s) Evaluated/ Duration	Sample Size/ Population	Results relating *Lactobacillus* colonization to antibiotic use
Baeten (2009)[14]	Prospective cohort	Self-reported use: Any antibiotic use, excluding metronidazole, last 60 days Penicillin: NA Macrolide: NA Fluoroquinolone: NA Tetracycline: NA Other: NA	N=1020 women (Kenyan) -18-45 years -HIV-seronegative sex worker	Recent antibiotic use: Reduced *Lactobacillus* colonization by 30% Strengths: Large sample size, antibiotic exposure categorized by class, *Lactobacillus* colonization assessed by culture Limitations: Cohort homogenous with respect to demographics and high risk sexual behaviors which limit generalizability
Vallor (2001)[25]	Prospective cohort	Self-reported use: Any antibiotic use last 4 months Penicillin: NA Macrolide: NA Fluoroquinolone: NA Tetracycline: NA Other: NA	N=101 women -14-44 years -Attending adolescent and STD clinics	Recent antibiotic use: Women more likely to lose colonization by hydrogen peroxide-producing lactobacilli Strengths: Large sample size, *Lactobacillus* colonization assessed by culture Limitations: More than half of initial cohort lost-to-follow-up, analyses not adjusted for potential confounding variables, prevalence of antibiotic exposure not reported
Agnew (1995)[26]	Prospective cohort	Self-reported use: NA Penicillin: Ampicillin, 7 days Macrolide: Azithromycin, 1 day Fluoroquinolone: NA Tetracycline: Doxycycline, 7 days Other: Clindamycin, 7 days Metronidazole, 5-7 days	N=165 women with vaginitis/cervicitis -18-45 years -Non-pregnant -No antibiotics <2 weeks	Ampicillin: -Increase in hydrogen peroxide-producing *Lactobacillus* at 1 week -Increased colonization at 1-month visit Azithromycin: -Increase in any *Lactobacillus* at 1 month -Decrease in hydrogen peroxide producing *Lactobacillus* Doxycycline: -Decrease in hydrogen peroxide-producing lactobacilli at 1 week -Return to pre-treatment levels by 1-month Clindamycin: -Decrease in *Lactobacillus* colonization at 1-week -Increase above pre-treatment levels at 1-month Metronidazole: Increase in *Lactobacillus* colonization Strengths: Several classes of antibiotics evaluated, *Lactobacillus* colonization assessed by culture Limitations: Small sample size within antibiotic class, statistical analyses not performed
Tempera (2009)[27]	Open-label randomized repeated dose study	Self-reported use: NA Penicillin: Amoxicillin, 5 days Macrolide: NA Fluoroquinolone: Prulifloxacin, 5 days Tetracycline: NA Other: NA	N=30 women -18-45 years -Non-pregnant -Normal menses -Normal vaginal flora -No antibiotic use <8 weeks	Prulifloxacin: No change in *Lactobacillus* colonization Amoxicillin: Decrease in *Lactobacillus* colonization Strengths: Randomized study design Limitations: Small sample size, *Lactobacillus* colonization assessed by vaginal smear

Table 2.1 (continued)

Sullivan (2005)[28]	Prospective open-label pilot trial	Self-reported use: NA Penicillin: Pivmecillinam, 7 days Macrolide: NA Fluoroquinolone: NA Tetracycline: NA Other: NA	N=20 women -24-40 years -Normal menses -No antibiotics <3 months	Pivmecillinam*:* No change in hydrogen peroxide-producing *Lactobacillus* at all visits Strengths: *Lactobacillus* colonization assessed by culture Limitations: Small sample size, statistical analyses not performed
Sjöberg (1992)[29]	Prospective open-label pilot trial	Self-reported use: NA Penicillin: Phenoxymethyl-penicillin, 10 days Macrolide: NA Fluoroquinolone: NA Tetracycline: NA Other: NA	N=6 women -Not using contraceptives -Normal vaginal flora for 2 menstrual cycles	Phenoxymethyl-penicillin: -No change in lactobacilli in 5 of 6 women -One woman lost colonization Strengths: *Lactobacillus* colonization assessed by culture Limitations: Small sample size, statistical analyses not performed, selective inclusion criteria
Kurowski (2000)[30]	Prospective cohort	Self-reported use: NA Penicillin: NA Macrolide: Clarithromycin, 7-10 days Fluoroquinolone: NA Tetracycline: NA Other: NA	N=12 women -18-55 years -Diagnosis of sinusitis or bronchitis -No conditions that affect vaginal flora	Clarithromycin: Decrease in *Lactobacillus* colonization Strengths: *Lactobacillus* colonization assessed by culture Limitations: Small sample size, statistical analyses may have been improperly performed
Cai T (2009)[31]	Prospective open-label pilot trial	Self-reported use: NA Penicillin: NA Macrolide: NA Fluoroquinolone: Prulifloxacin, 14 days Tetracycline: NA Other: NA	N=51 women -18-45 years -Symptomatic UTI -History of recurrent UTI -Non-pregnant -Negative for sexually transmitted infections	Prulifloxacin: No change in *Lactobacillus* colonization Strengths: *Lactobacillus* colonization assessed by culture Limitations: Small sample size, 1- and 3-month post-treatment results not presented
Moore (2000)[32]	Prospective clinical trial	Self-reported use: NA Penicillin: NA Macrolide: NA Fluoroquinolone: NA Tetracycline: Doxycycline, 5 days Other: NA	N=91 women -21-45 years -Undergoing *in vitro* fertilization	Doxycycline: No change in hydrogen peroxide-producing *Lactobacillus* Strengths: *Lactobacillus* colonization assessed by culture Limitations: Paired statistical analyses not performed

NA, not assessed

2.3 ASSOCIATION OF ANTIBIOTIC USE WITH VULVOAGINAL CANDIDIASIS

Of 9 studies published within the last twenty years which evaluated the association between antibiotic exposure and vulvovaginal candidiasis (VVC) in non-pregnant women, two were prospective cohort studies, four were case-control studies, two were cross-sectional, and one was a randomized clinical trial (Table 2.2). Eight studies evaluated antibiotic use as a dichotomous variable, four of these included some analysis of antibiotic use by class, and one only evaluated metronidazole. For the nine studies, four reported a statistically significant positive association between antibiotic use and VVC, while the remaining five found no significant association.[36-44] These studies are described in more detail below.

Three prospective and one retrospective studies reported an association between antibiotic use and increased VVC.[40-43] A randomized clinical trial evaluated incident VVC within 28 weeks of follow-up among women who received 10 days of metronidazole therapy for treatment of bacterial vaginosis. Of the 112 women whose bacterial vaginosis had resolved after the initial antibiotic regimen, half were randomized to a suppression regimen of metronidazole administered twice weekly and half to placebo. Women with history of recurrent gynecological infection, including VVC, were excluded. Since it was a secondary outcome, the diagnostic criteria for VVC were not described. The incidence of VVC was twice as high among women in the metronidazole group (43.1%) than among women in the placebo group (20.5%) which was a statistically significant difference. No other antibiotics were evaluated in this study.[40] The major strength of this study was women were randomized to the exposure of interest and the antibiotic regimen was uniform. The limitations of this study were the lack of adjustment for other possible risk factors variables and the lack of generalizability of results since all women initially had bacterial vaginosis and had resolution of symptoms prior to randomization.

A small prospective matched cohort study evaluated incident symptomatic VVC, determined by clinical examination and diagnostic tests, and prevalence of positive vaginal *Candida* cultures in 44 women who were prescribed at least a 3-day course of antibiotics for a non-gynecologic diagnosis and 36 age-matched women with non-infectious conditions who did not receive antibiotics. The outcomes were evaluated at 1-2 weeks and 4-6 weeks. None of the women who did not receive antibiotics developed VVC and 11% had positive *Candida* cultures at follow-up while 22% and 37% of women who received antibiotics were positive for these outcomes, respectively. After adjusting for baseline *Candida* colonization, race, smoking, and oral sex, any antibiotic use was associated with nearly a 5- and 8-fold increased risk of VVC or positive follow-up vaginal *Candida* culture. The prospective nature of this study and uniform duration of antibiotic exposure among women recruited were strengths of this study. However, not only was the initial sample size small, but there was a 33% attrition rate in this study so the risk associated with antibiotic use may have been overestimated.[41]

A large prospective cohort study evaluated the seven week incidence of VVC in 77,080 women receiving prescriptions for one of six antibiotics or one of six antidepressants identified from a post-marketing surveillance database. Diagnosis of VVC was determined by reports submitted by general practitioners. Overall, the incidence of VVC was less than 1%, in both groups of women, which is less than that observed in the other studies included in this review. VVC was 4 times more likely to occur among women taking antibiotics than those taking antidepressants, after adjusting for age. The unadjusted relative risk for VVC did not vary between women taking fluoroquinolones, cephalosporins, or macrolides.[42] In addition to the rather large sample size, another strength of the study was the lack of inclusion criteria other than receiving a prescription for one of the drugs included in the study so the results are likely

generalizable to other populations. A major limitation of this study was the extremely low incidence of VVC. It was likely that there was under-ascertainment of VVC since detection of events relied on clinicians submitting study reports and the diagnostic criteria likely varied between clinicians.

A large case-control study evaluated the association between prevalent symptomatic VVC and any and class-specific antibiotic use. This study included 684 women with symptomatic VVC, defined as presence of symptoms and positive *Candida* culture, and 901 asymptomatic controls. The prevalence of antibiotic use in the prior month was 19% among women with VVC and 11% among the asymptomatic controls. While there was an adjusted 2-fold increased risk of incident VVC among women reporting any antibiotic use within the prior month compared to women not using antibiotics, this association did not vary by specific classes of antibiotics (penicillins, cephalosporins, fluoroquinolones, or sulfonamides). Antibiotic use was also associated with an increased risk of a repeated episode of VVC. Using antibiotics for more than 3 days was also significantly associated with VVC while a shorter course of antibiotics was not which suggested a dose-response relationship between duration of antibiotic exposure and VVC. The use of hormonal contraception and intrauterine devices were also associated with a 2-fold increased risk of VVC compared to women not using birth control. Sexual behaviors, including new sexual partner and frequency of vaginal intercourse, were not associated with VVC in this study. The strengths of this study included the large sample size, the diagnosis of VVC was based on symptoms and confirmed by culture, categorizing antibiotic use by class, and being able to establish a slight dose-response relationship. A limitation of this study was that the cases and controls were recruited from separate clinics so there may have been characteristics common to the cases that predisposed them to VVC.[43]

Two studies that evaluated antibiotic use as a dichotomous variable and by specific classes found no association with VVC. A prospective cohort study followed 151 asymptomatic, HIV-seronegative sex workers in Kenya for 12 months. These women received an oral and intravaginal placebo study product at enrollment. Symptomatic and asymptomatic VVC, defined as detection of yeast by wet mount microscopy, was present at 10% of 1,570 visits. Antibiotic use was reported at 12% of visits among women both with and without VVC. There was also no association with VVC when antibiotic use was stratified by metronidazole and all other classes. This study also did not find an association between VVC and hormonal methods of contraception (oral contraceptives, depot medroxyprogesterone acetate, intrauterine devices, or injectables) or sexual behavior measured by new or numbers of sexual partners. Concurrent vaginal *Lactobacillus* colonization was associated with an adjusted 4-fold increased risk only among the subset of women without concurrent bacterial vaginosis.[44] While the characteristics of the cohort included in this study limits the generalizability of the results to other populations, the strengths of this were the prospective cohort design and evaluation of class-specific antibiotic use. The second study compared 64 women with symptomatic VVC confirmed by positive *Candida* culture to two different control groups; 196 asymptomatic women attending a university health clinic and 431 respondents to a mailed survey. Overall or class-specific antibiotic use was not associated with VVC, although the prevalence of antibiotic use was not reported. Oral contraceptives, vaginal intercourse or number of sexual partners were also not associated with VVC in adjusted analyses. However, women who engaged in receptive oral sex 2 or more times in the last 2 weeks were 3 times more likely to have VVC compared to women denying oral sex.[45] This study may have lacked statistical power to detect an association between antibiotic use, especially class-specific antibiotic use, due to the small number of cases.

The three remaining studies included in this review found no association between any antibiotic use and VVC. A prospective cohort followed 316 women, who denied antibiotic use in the past 30 days and were asymptomatic at enrollment, for up to 4 years. VVC, defined as use of an antifungal, was reported by 46 (14.5%) women and there were 488 reported prescriptions of antibiotics. Overall, 29% of women who used an antibiotic also used an antifungal compared to 19% antifungal use among women who did not use an antibiotic. This difference was not statistically different. The authors also found no association between VVC and time since antibiotic use.[46] Limitations of this study were that VVC may have been over-ascertained since antifungal use was used as a surrogate for a definitive diagnosis and the use of a rural clinic population limits the generalizability of results.

Two cross-sectional studies also reported no association between antibiotic use and prevalence of positive vaginal *Candida* cultures. One study that evaluated 774 women who presented to the clinic with a gynecological problem, reported a 24% prevalence of a positive *Candida albicans* culture. The prevalence of antibiotic use 15 to 30 days prior to the vaginal culture was 6% among culture negative women and 13% among culture positive women. The effect of antibiotic use that was closer to the time of culture could not be evaluated since women were excluded if they had used an antibiotic in the past 14 days. There was also no association between the use of oral contraceptive pills or vaginal sexual intercourse and the presence of positive *Candida* cultures. However, women with concurrent vaginal *Lactobacillus* colonization were 80% more likely to have a positive *Candida albicans* culture.[47] Using a positive *Candida* cultures as the outcome likely resulted in capturing both symptomatic and asymptomatic VVC since 10% of women are asymptomatically colonized by yeast in the vagina.[35] The second study evaluated 219 sexually active adolescents, aged 12 to 22 years, who presented at a health clinic

for a gynecological examination. Antibiotic use was not associated with the prevalence of a positive *Candida* culture which was 42%. However, antibiotic use was defined as more than 3 times in the past 2 years so temporal associations of antibiotic use with vaginal *Candida* were not assessed. Oral sex was associated with an increased prevalence of positive *Candida* culture in this population, however frequency of vaginal intercourse, number of sexual partners, and use of oral contraceptives were not.[48] Limitations of this study were that both symptomatic and asymptomatic VVC were captured and the results are likely not generalizable to adult women.

While its etiology is complex and unknown, it is widely believed by clinicians and women that antibiotic use is a risk factor for developing VVC. However, the results of the nine studies included in this review that evaluated antibiotic use in non-pregnant women show there is very little evidence to support this view, and more than half of these studies found no association between antibiotic use and VVC. There was heterogeneity in these studies with respect to the definitions of the outcome and antibiotic use, study design, comparator groups, and adjustment for other possible risk factors. Studies that evaluated a positive vaginal *Candida* culture as the outcome captured both asymptomatic colonization and symptomatic VVC which may have influenced the magnitude of the association of antibiotic use on VVC. Large prospective studies with detailed information on antibiotic use by class and duration that is also proximal to the diagnosis of VVC may be able to better assess the association between antibiotic use and VVC.

Table 2.2 Characteristics of studies evaluating antibiotic use for vulvovaginal candidiasis in non-pregnant women

Author	Study Design	Outcome/ Sample Size/ Population	Risk Factors Evaluated	Results
Sobel (2006)[40]	Randomized control trial	VVC within 28 weeks N=112 women with resolved bacterial vaginosis after 10 days of metronidazole therapy -56 received metronidazole suppressive therapy -56 received placebo -Women with history of recurrent gynecological infection excluded	Antibiotic use: -Metronidazole	Incident VVC associated with metronidazole use: 43.1% in metronidazole arm, 20.5% in placebo arm Strengths: Randomized study design, uniform antibiotic regimen Limitations: No adjustment for other possible risk factors, limited generalizability of results
Xu (2008)[41]	Prospective matched cohort	Incident symptomatic VVC (by exam and diagnostic tests) within 4-6 weeks Prevalence of positive *Candida* culture at 1-2 weeks and 4-6 weeks N=80 women: -44 prescribed ≥3 days of systemic antibiotics for non-gynecological diagnosis -36 age-matched (±5 years) women with non-infectious conditions who did not receive antibiotics -18-64 years -No antibiotics/antifungals in past 4 weeks	Any antibiotic use	VVC: antibiotic use adjusted odds ratio = 4.8 Positive *Candida* culture: antibiotic use adjusted odds ratio = 7.8 Strengths: Prospective study design, uniform duration of antibiotic exposure Limitations: Small sample size; high attrition rate (n=27 women)
Wilton (2003)[42]	Prospective cohort	VVC within 7 weeks (by clinician report) N=77,080 women -31,588 treated with antibiotics -45,492 treated with antidepressants -Age ≥16 years	Antibiotic use, any and class-specific	Any antibiotic use; adjusted odds ratio = 3.9 Relative risks were similar for cephalosporins, macrolide, and fluoroquinolones Strengths: Prospective study design, large sample size, generalizability of results Limitations: Unknown, possibly variable diagnostic criteria, possible under-ascertainment of VVC (incidence <1%)
Spinillo (1998)[43]	Case-control	Prevalent symptomatic VVC (presence of symptoms and positive *Candida* culture) N=1585 women; -684 with symptomatic VVC -901 asymptomatic controls	Antibiotic use, any and class-specific, duration Hormonal contraception Intrauterine device Sexual behavior	Any antibiotic use, previous month: adjusted odds ratio = 1.75 Class-specific, unadjusted odds ratio: -Penicillins =1.93; Cephalosporins =1.73; Fluoroquinolones =1.89 -Sulfonamide = 2.34; Others = 1.31 (not significant) Duration of antibiotic use >3 days, unadjusted odds ratio = 1.97 Duration of antibiotic use 1-3 days, unadjusted odds ratio = 1.50 (not significant) Hormonal contraception: unadjusted odds ratio = 2.5 Intrauterine device: unadjusted odds ratio = 2.2 Sexual behaviors (new partner, frequency of vaginal intercourse) not associated Strengths: Large sample size, evaluation of class-specific antibiotic use; dose-response relationship Limitations: Cases and controls recruited from separate clinics-possible selection bias

Table 2.2 (continued)

McClelland. (2009)[44]	Prospective cohort	Symptomatic and asymptomatic VVC (presence of yeast by wet mount microscopy) within 12 months N=151 women -Sex workers in Kenya -18-45 years -HIV seronegative -Asymptomatic -Received placebo oral and intravaginal study product	Antibiotic use, metronidazole, all others Hormonal contraception Sexual behavior Concurrent vaginal *Lactobacillus* colonization	Antibiotic use not associated Hormonal contraception (oral contraceptives, DMPA, intrauterine device, injectable) not associated Sexual behavior (number of partners, new partner) not associated Concurrent vaginal *Lactobacillus* colonization: adjusted odds ratio = 3.75 only among subset without concurrent bacterial vaginosis Strengths: Prospective study design, evaluation of class-specific antibiotic use Limitations: Cohort homogenous with respect to demographics and high risk sexual behaviors which limit generalizability
Geiger (1995)[45]	Case-control	Symptomatic VVC (confirmed by positive *Candida* culture) N=691 women -64 cases -196 asymptomatic controls -431 respondents from mailed survey	Antibiotic use, any and class specific Oral contraceptives Sexual behaviors	Antibiotic use , any or class-specific, not associated Oral contraceptives not associated in adjusted analyses Receptive oral sex, $\geq$2 times in last 2 weeks: adjusted odds ratio =3.5 Vaginal intercourse or number of sexual partners not associated Strengths: Evaluation of class-specific antibiotic use Limitations: Small number of cases
Glover (2003)[46]	Prospective cohort	Incident VVC by use of an antifungal within 4 years N=316 women -Asymptomatic -No antibiotics past 30 days	Any antibiotic use	No association with VVC Strengths: Prospective study design Limitations: Diagnostic criteria – possible over-ascertainment of VVC; limited generalizability
Eckert (1998)[47]	Cross-sectional	Positive *Candida albicans* culture N=774 women -Age 16-50 years -Presenting with a gynecological problem -No antibiotics past 14 days	Any antibiotic use Hormonal contraception Sexual behavior Concurrent vaginal *Lactobacillus* colonization	Any antibiotic use, 15-30 days prior to culture, not associated in adjusted model Oral contraceptive pills not associated Vaginal sexual intercourse (>4 times/month) not associated in adjusted model Concurrent vaginal *Lactobacillus* colonization: unadjusted odds ratio = 1.8, adjusted odds ration not shown Strengths: Large sample size Limitations: Both symptomatic and asymptomatic VVC captured
Rylander (2004)[48]	Cross-sectional	Prevalence of positive *Candida* culture N=219 women -12-22 years -Sexually active -Presenting for genital examination	Any antibiotic use Oral contraceptives Sexual behaviors	No association with antibiotic use (>3 times in past 2 years), oral contraceptives, frequency of vaginal intercourse, number of sexual partners Oral sex: increased prevalence of positive *Candida* culture Limitations: Both symptomatic and asymptomatic VVC captured, unable to assess temporal associations; limited generalizability

VVC, vulvovaginal candidiasis; DMPA, depot medroxyprogesterone acetate; HIV, human immunodeficiency virus

2.4 SUMMARY

Antibiotic use is widespread and inappropriate use remains a public health concern despite national campaign efforts promoting judicious and proper antibiotic prescribing practices. Furthermore, very little is known about the burden of antibiotic use among healthy, non-pregnant women. Antibiotics could impact beneficial members of the vaginal flora such as *Lactobacillus* and may affect colonization by potential pathogens like *Candida spp*. The impact of antibiotic usage could occur directly in the vaginal microenvironment or in the rectal reservoir. To date, studies that evaluated the effect of antibiotic use on colonization by *Lactobacillus* and developing vulvovaginal candidiasis have mostly included crude assessments of antibiotic use or evaluated a single antibiotic in a controlled study. These studies have also included selective populations ranging from healthy women to those presenting with genital infections to commercial sex workers. Thus, the generalizability of the results is limited. Studies have not been designed to evaluate the relationship between antibiotic use and rectal *Lactobacillus* colonization. Similarly, while there is ample evidence that the *in vitro* susceptibility of *Lactobacillus* to specific antibiotic classes varies by species, studies have not evaluated whether any association between antibiotic use and colonization by these organisms differs between species. Such studies could provide valuable information that can be used in guiding choices for antibiotic therapy or prophylaxis in target populations such as pregnant women, women at high risk for acquiring sexually transmitted infections or with a history of recurrent vulvovaginal candidiasis, immunocompromised adults, or the elderly.

The following analyses are derived from a longitudinal evaluation of overall antibiotic use and class-specific antibiotic use in young, sexually active women. The frequency, type, and indication for antimicrobial use in healthy, non-pregnant women are described along with

demographic and behavioral factors associated with increased antibiotic use. In addition, the associations of overall and class-specific antibiotic use with the prevalence of vaginal and rectal colonization by *Lactobacillus* and the incidence of vulvovaginal candidiasis are also presented.

3.0 SPECIFIC AIMS AND POWER ANALYSES

This dissertation is composed of three manuscripts, each pertaining to systemic and/or intra-vaginal antibiotic use in non-pregnant women.

Specific Aim for Manuscript 1:

Describe the rate of antimicrobial use in healthy, non-pregnant women, identify the classes of antibiotics used, indications for use, estimate the amount of possible inappropriate use by consulting current prescribing recommendations, and identify demographic and behavioral factors associated with antibiotic use. This paper will improve our knowledge as to the burden of and reasons for antibiotic use in reproductive-aged women that may be used to help tailor educational efforts to promote judicious use of antibiotics in this population.

Hypothesis: There are no differences in women who use or do not use antibiotics.

Alternative Hypothesis: There will be differences in race and sexual behaviors between women who do and do not use antibiotics.

Power Analysis for Specific Aim 1: The marginal prevalence of antibiotic use in this cohort was 10.8%. Based on a Fisher's exact test evaluated at the 2-sided 0.05 significance level, this study will have 80% power to detect at least a 75% difference in the prevalence of antibiotic use between women who do and do not have the exposure of interest.

Specific Aim for Manuscript 2: Evaluate the association of any and class-specific antibiotic use on vaginal and rectal colonization by hydrogen peroxide-producing *Lactobacillus spp.* This manuscript will be the first to evaluate the association of antibiotic use on rectal colonization by

lactobacilli and could improve our knowledge as to whether the use of any or only specific classes of antibiotics disrupts vaginal *Lactobacillus* colonization.

Hypothesis: There will be no difference in the prevalence of vaginal or rectal *Lactobacillus* colonization between women with recent antibiotic exposure and those who do not have recent antibiotic exposure.

Alternative Hypothesis: Women exposed to some classes of antibiotics will have a lower prevalence of vaginal or rectal *Lactobacillus* colonization than women without recent antibiotic exposure.

Power Analysis: Based on a Fisher's exact test evaluated at the 2-sided 0.05 significance level, this study will have 85% power to detect at least a 20% difference in the prevalence of *Lactobacillus* colonization between women who do and do not use antibiotics, assuming the prevalence in the unexposed group is at least 0.6.

Specific Aim for Manuscript 3:

Evaluate the association between any and class-specific antibiotic use and acquisition of vulvovaginal candidiasis in healthy, non-pregnant women. This paper could improve our knowledge pertaining to the association of overall antibiotic use with increased incidence of vulvovaginal candidiasis and whether the association varies by antibiotic class.

Hypothesis: Recent antibiotic use will not be associated with incident vulvovaginal candidiasis

Alternative Hypothesis: Women who report recent antibiotic use will be more likely to develop vulvovaginal candidiasis

Power Analysis: Based on previous studies[40-43], women exposed to antibiotics have at least a 75% increased risk of developing vulvovaginal candidiasis. This study will have 80% power to

detect at least a 60% difference in the risk of acquiring vulvovaginal candidiasis between women who do and do not use antibiotics, based on a log-rank test for equality of survival curves evaluated at the 2-sided 0.05 significance level.

4.0 MANUSCRIPT 1: LONGITUDINAL EVALUATION OF ANTIBIOTIC USE IN NON-PREGNANT, REPRODUCTIVE-AGED WOMEN

To be submitted for publication

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4.1 ABSTRACT

Objective: Describe antibiotic use patterns and indications for use among healthy non-pregnant women, and evaluate factors associated with antibiotic use.

Study Design: 650 women, aged 18-40 years, were followed bi-monthly for up to 18 months in a prospective cohort study conducted in Pittsburgh, PA, Augusta, GA, and Houston, TX. Behavioral and antimicrobial use information was collected by interview. Poisson regression analysis was used to evaluate risk factors for antibiotic use. Appropriate antibiotic use was assessed using CDC recommendations for treatment of upper respiratory infections (URI).

Results: The antibiotic use rate was 81.2 per 100 woman-years (95% confidence interval (CI): 75.4-87.5). Of the 699 episodes of antibiotic use, the most common classes of antibiotics were metronidazole (24.2%), penicillins (22.6%), and macrolides (15.2%); 32.5% were broad spectrum agents. Treatment of genitourinary infections accounted for 48.5% of all antibiotic use, while URI accounted for 26.2%. Of the 183 antibiotic uses for URI, 104 (56.8%) were broad spectrum antibiotics. Bronchitis, rhinosinusitis, and non-specific URI accounted for 21.2% of the total and 40.5% of the broad spectrum antibiotic prescriptions. Older age and being white were associated with inappropriate antibiotic use. Factors independently associated with antibiotic use, excluding those used for the treatment of URIs, included black race, increasing number of sex partners, and reduced vaginal lactobacilli.

Conclusion: The high antibiotic use burden in this population of young women was likely appropriate for the primary indications of genitourinary infections. However, broad-spectrum agents were often selected to treat URI conditions for which antibiotics provide little therapeutic benefit.

4.2 INTRODUCTION

Antibiotics are one of the most commonly prescribed classes of medications. Widespread antibiotic use in humans and in commercial agriculture has contributed to a new public health threat, the emergence of antibiotic resistant bacteria. The Centers for Disease Control and Prevention estimate that antibiotic resistant infections cause more than 2 million illnesses and 23,000 deaths per year in the United States.[3] Hence, promoting appropriate antibiotic use is a public health priority.[9] Understanding antibiotic prescribing patterns can help direct educational efforts to reduce unjustified antibiotic use.

While antibiotic prescribing patterns have been frequently described in outpatient and inpatient hospital populations, children, and the elderly, there are few reports about antibiotic use specifically in reproductive-aged women.[50, 52-55] A study of antibiotic prescribing patterns in general practices in the United Kingdom, reported that the overall antibiotic prescribing rate in females, including children, was 852 per 1000 patient years in 1996 which was higher than the rate in males (607 per 1000 patient years). However, the antibiotic prescribing rates in women 16-34 years of age was nearly twice as high as the rate in men in the same age group.[56] A study which evaluated 115,000 women who had a live birth between 1992 and 2007 in the United Kingdom, reported that antibiotic prescriptions for respiratory and urinary tract infections in the year prior to pregnancy were 269 and 93 per 1000 women, respectively. While the antibiotic prescribing rate for respiratory infections during pregnancy was similar to the pre-pregnant rate, the prescribing rate for urinary tract infections nearly doubled during pregnancy.[58] The results of these studies indicate that antibiotic consumption is considerably higher in reproductive-aged women than in men of the same age, and that the indications for use and subsequently the classes of antibiotics prescribed change during pregnancy.

The current literature is lacking in the epidemiology of antibiotic use in non-pregnant, reproductive-aged women, particularly in the US which has one of the highest antibiotic consumption rates.[52] Sexually active women are at risk for acquiring an infection by antibiotic resistant bacteria such as drug resistant *Neisseria gonorrhoeae*, which is classified as an urgent public health threat in the US.[1] Studies on antibiotic prescribing patterns in non-pregnant women are needed in order to understand the burden of antibiotic use in this population and to help tailor educational efforts to promote judicious use of antibiotics in this population.

The primary objectives of this longitudinal study were to describe antibiotic use patterns in non-pregnant, reproductive-aged women and to describe the classes of antibiotics and the indications for which they were prescribed. Another objective was to evaluate demographic and behavioral risk factors for antibiotic use.

4.3 METHODS

4.3.1 Study population

This is a secondary analysis of a randomized, clinical trial designed to evaluate the efficacy of a group B *Streptococcus* (GBS) serotype III polysaccharide-tetanus toxoid conjugated vaccine. Briefly, non-pregnant, sexually active women aged 18-40 years who were using effective birth control methods, denied antimicrobial and antifungal use in the past 7 days, and were GBS culture-negative both vaginally and rectally were recruited from primary care and family planning settings in Pittsburgh, PA, Augusta, GA, and Houston, TX. Participants (n=663) were randomized to receive a single dose of GBS type III- tetanus toxoid vaccine or a licensed vaccine containing tetanus and diphtheria toxoids adsorbed for adult use. The clinical trial was approved

by the institutional review boards of the participating institutions. All participants provided written informed consent. The trial enrollment occurred from July 2003 to August 2006, and all follow-up visits were completed by February 2008.

Vaginal swabs for culture detection of GBS and lactobacilli, a vaginal smear, demographics, behavioral information, and use of systemic or intra-vaginal antimicrobials were collected by pelvic examination and interview administered by trained research personnel at enrollment, 1- and 2-months, and then bi-monthly for 18 months. Antibiotic use was categorized by antibiotic class. The following classes of antibiotics were classified as broad-spectrum agents: amoxicillin/clavulanate, second- and third-generation cephalosporins, macrolides, fluoroquinolones, and lincosamides. GBS and *Lactobacillus* were isolated and identified by methods that have been previously described.[76] The vaginal smear was Gram-stained and evaluated for bacterial vaginosis according to the Nugent criteria.[77]

4.3.2 Covariates

Variables considered as potential explanatory factors included study site, study arm, race, age, education, and marital and employment status at enrollment. Other variables which could change over time and for which data was collected at each visit included number of male sexual partners, new sex partner, frequency of vaginal intercourse, receptive anal intercourse, douching, Nugent score, vaginal *Lactobacillus* and GBS colonization, and contraceptive methods. Contraceptives were categorized as none, non-barrier methods (spermicides, tubal ligations, or copper intrauterine devices), barrier methods (condoms, diaphragms, or cervical caps), hormonal methods (oral contraceptive tablets, intrauterine devices containing levonorgestrel, the vaginal ring, transdermal patches, or implants), and depot medroxyprogesterone acetate (DMPA).

4.3.3 Statistical analysis

Statistical analyses were performed using Stata statistical software release 11.2 (Stata Corp., College Station, TX), and statistical tests were evaluated at the 0.05 two-sided significance level. All women who completed at least one follow-up visit (N=650) were included in the analyses. The antibiotic use rate represents the number of antibiotic prescriptions per 100 woman-years. A population averaged Poisson longitudinal regression model based on repeated visit measures per woman was used to evaluate risk factors for antibiotic use and estimate the population averaged incidence rate ratios (IRRs). The count of antibiotic prescriptions that were reported since the previous visit up to the current visit for a given woman was the Poisson outcome variable, and the length of time from the previous visit to the current visit was the offset term. An exchangeable working correlation matrix was specified and modified sandwich estimates of the variance were calculated.[78] Multivariable models were developed using forward regression and variables were retained in the model if the *P* value from the Wald chi-squared test statistic was 0.05 or less. The final model was also adjusted for age. Appropriate antibiotic use was assessed using CDC recommendations for treatment of upper respiratory infections (URI).[79] A modified Poisson regression model with sandwich estimates of the variance was used to identify factors that were associated with receiving any antibiotic for the subset of URI for which the use of antibiotics was inappropriate (bronchitis, rhinosinusitus, and non-specific URI) and to estimate relative risks.[80] Models were developed using the same aforementioned procedure.

4.4 RESULTS

This analysis included 650 women who completed 5599 follow-up visits over 860.8 woman-years. Most women (60%) completed all 10 scheduled follow-up visits and 91% completed 5 or more visits; the median follow-up time was 1.5 years. Antimicrobial use was reported by 341 (52.5%) women at least once and one or more new antibiotic prescriptions were reported at 599 (10.7%) of the follow-up visits. Women reported using a total of 699 antibiotic prescriptions for an overall antibiotic use rate of 81.2 per 100 woman-years of follow-up (95% confidence interval (CI): 75.4-87.5). Prescriptions for more than one course of antibiotics was reported at 75 visits; 2 courses at 52 visits, 3 at 21 visits, and 4 at 2 visits. Among the women who reported antimicrobial use, the median number of prescriptions was 2 (range: 1-9 prescriptions). The most commonly reported antibiotics used were metronidazole (24.2%), penicillins (22.6%), and macrolides (15.2%), followed by fluoroquinolones (8.7%), cephalosporins (8.2%), sulfonamides (6.7%), and tetracyclines (6.0%). The name of the antibiotic used could not be verified for 30 (4.3%) of the prescriptions reported (Table 4.1). Of the 699 prescriptions reported by women, 227 (32.5%) were for broad spectrum antibiotics.

The indications for antibiotic use by classes of antibiotics are shown in Table 4.2. Almost half (48.5%) of antibiotics were used to treat genitourinary conditions in 188 women. Bacterial vaginosis accounted for 20.6% of all antibiotic use, while treatment of urinary tract infections accounted for 13.3%, and sexually transmitted infections, including trichomoniasis, chlamydia, and gonorrhea, accounted for 10.9% of all antibiotic use. Genitourinary infections accounted for nearly all of the metronidazole use (96.4%), 62.3% of the fluoroquinolone prescriptions, and 53.5% of prescriptions for tetracyclines, sulfonamides, nitrofurantoin, lincosamides, aminoglycosides, and unknown antibiotics. About a third of cephalosporin (38.6%) and

macrolide (28.3%) prescriptions were also for genitourinary infections, primarily for the treatment of sexually transmitted infections.

Antibiotics were prescribed for 176 of the 380 (46.3%) upper respiratory infections that were reported to study personnel by 131 women and accounted for 26.2% of the antibiotics used. Two courses of antibiotics were prescribed for seven URIs for a total of 183 antibiotic uses and 104 (56.8%) of those were broad spectrum antibiotics. Pharyngitis cases that were presumed to be due to group A streptococcal infection and pneumonia accounted for 19.1% of the URI antibiotic uses, bronchitis and rhinosinusitis for 53.0%, and the remaining 27.9% were non-specific URI. Bronchitis, rhinosinusits, and non-specific URI accounted for 21.2% of the total and 40.5% of the broad spectrum antibiotic use. URI contributed to more than half of the exposure to macrolides (59.4%) and penicillins (51.3%) in this cohort of women.

There were 340 cases of bronchitis, rhinosinusitus, or non-specific URI reported by 229 women and 143 (42.1%) of the cases among 106 women were treated with an antibiotic; among the antibiotics prescribed, 88 (60.5%) were broad-spectrum agents and 74 (50.0%) were β-lactams (penicillins, cephalosposrins). Older age and being white were independently associated with receiving an antibiotic for bronchitis, rhinosinusitus, or non-specific URI, which was considered to be inappropriate antibiotic use (Table 4.3). White women were more likely to receive an antibiotic for these conditions than non-white women (adjusted relative risk (aRR) =1.51, 95% confidence interval (CI): 1.08-2.10), as were women 35-40 years of age compared to 18-19 year olds (aRR=2.42, 95% CI: 1.05-5.57).

Following genitourinary infections and URIs, the next most common indications for antibiotic use were dental (9.6%) and skin conditions (8.0%). Antibiotic use for other indications (gastrointestinal, ear and eye infections, musculoskeletal, and others) was uncommon (7.7%).

Dental indications, which included dental infections, abscesses, and prophylaxis for cleaning, extractions and surgery, accounted for 32.9% of the penicillin use. Most of the skin conditions were treated with cephalosporins (30.4%) or other classes of antibiotics (55%) which were primarily tetracyclines for the treatment of acne (Table 4.2).

The antibiotic use rate did not vary significantly by vaccine arm, employment and marital status, or methods of contraception. While the antibiotic use rates at the Pittsburgh and Houston study sites were similar (92.8 and 97.6 per 100 woman-years, respectively), the antibiotic use rate was significantly lower at the Augusta site, at 49.5 per 100 woman-years. Antibiotic use also varied significantly by categories of race, age, and education. Black women used more antibiotics than white women (94.3 versus 68.0 uses per 100 woman-years), as did women without any college education compared to women with at least 17 years of education (100.3 versus 60.9 per 100 woman-years). Antibiotic use also varied across the age spectrum with the lowest rates in teens (58.4), increasing among women in their early twenties, peaking in 25-29 year olds at 93.3, and then declining to 62.1 among women 35-40 years of age (Table 4.4).

Sexual activity was associated with antibiotic use. Women who reported a new sexual partner since the last visit and those reporting multiple male partners used antibiotics more often than women who did not report a new partner or were sexually abstinent. Vaginal microflora at the prior visit was also associated with antibiotic use rates. Women with a Nugent score $\geq$4, which reflects reduced numbers of vaginal lactobacilli, had higher antibiotic use rates than those with a score that is consistent with a *Lactobacillus*-predominant vaginal microflora (Table 4.4).

Multivariable regression models to determine correlates of appropriate antibiotic use were constructed after excluding antibiotics used to treat URIs (Table 4.5). Race, number of sex partners, abnormal vaginal microflora (Nugent score 4-10) at the prior visit, and study site were

independently associated with antibiotic use. The adjusted incident rate ratio (aIRR) for black women was 1.48 (95% confidence interval (CI): 1.15-1.90) compared to white women. Women reporting one sex partner had a 48% increased aIRR (95% CI: 1.03-2.14) and those reporting 2 or more partners a 2-fold increase (aIRR=2.04, 95% CI: 1.19-3.50), as compared to sexually abstinent women. A Nugent score $\geq$4 at the prior visit was associated with a 80% increased aIRR (95% CI: 1.45-2.24).

Compared to women at the Pittsburgh site, women at the Augusta site had a decreased aIRR of 0.49 (95% confidence interval (CI): 0.36-0.66), while women at the Houston site did not have a significantly decreased aIRR (Table 4.5). However, only 38.1% of women in Augusta were 25 years or older compared to 51.1% of women at the other two sites (p=0.003). Women in Augusta were also less likely to have a Nugent score $\geq$4 at their prior visit than women from the Pittsburgh and Houston sites (odds ratio=0.67, 95% CI: 0.51-0.87; p=0.003) and less likely to have multiple sex partners (p=0.050, data not shown). Since there were differences between the study sites in several factors associated with antibiotic use, site-specific analyses were performed. While number of sex partners and Nugent score $\geq$4 at the prior visit remained significantly associated with increased antibiotic use among women from Pittsburgh and Houston, black race was the only factor associated with antibiotic use for indications other than URI among women from Augusta.

4.5 DISCUSSION

The overall antibiotic use rate in this population of non-pregnant, reproductive-aged women from 2003 to 2008 was 81.2 per 100 woman-years. This rate is similar to the national average of 80.1 among all persons in the US in 2010.[49] However, the antibiotic use rate for the Pittsburgh study site of reproductive-aged women was 92.8 per 100 woman-years which is higher than the rate for the entire state of Pennsylvania in 2010 (78.7). The antibiotic use rate for the Houston site was also higher than the Texas state average (97.6 versus 86.7). The rate for the Augusta site, however, was considerably lower than the Georgia state average (49.5 versus 85.2). The national and state-specific antibiotic prescribing rates were based on the entire population whereas the antibiotic use rates in this study were based on only reproductive-aged women, which likely accounts for the differences since previous studies have shown that the antibiotic prescribing rates in women were higher than those of men.[56] Although antibiotic use was high in our study population, the rates observed were similar to those observed among women 16-34 years of age in a population based study in Wales.[56] The antibiotic use rate among women in Augusta was nearly half of the rate among women from Pittsburgh and Houston. However, this was likely due to differences in age, education, sexual activity, and Nugent score among women between the three study locations since these factors were also associated with antibiotic use in this study.

Metronidazole, penicillins, macrolides, fluoroquinolones, and cephalosporins were the five most commonly prescribed classes of antibiotics for the women in this study, which is similar to the antibiotic class distribution for the US in 2010 where the four most common antibiotics dispensed were penicillins, macrolides, cephalosporins, and fluoroquinolones.[49] The primary difference in the distribution of antibiotics used was for metronidazole, the most

prescribed antibiotic in the present study. Metronidazole was prescribed primarily for the treatment of bacterial vaginosis, the most common genital tract infection in our population of adult women. URI is the most common indication for antibiotic use in other adult populations.[10, 62]

In our study of young women, nearly half of the antibiotics were prescribed for genitourinary conditions, followed by URI, dental conditions or procedures, and skin conditions. In contrast, a study that evaluated women before and during their pregnancy, found that antibiotics were most often used to treat URIs, followed by genitourinary conditions. Antibiotics were also most often used to treat URIs, followed by genitourinary conditions, skin and soft tissue infections, and digestive conditions in studies that have evaluated adults in outpatient care settings and nursing homes.[10, 58, 62, 63]

Minimizing the inappropriate use of antibiotics is a public health priority.[9] Upper respiratory infections are a major target in national campaigns efforts to reduce unjustified antibiotic use since studies indicate a high prevalence for over-prescribing antibiotics for these conditions.[10, 62] Furthermore, the use of broad-spectrum antibiotics contributes to the development of antibiotic-resistant infections.[81, 82] In the present study, while 46% of upper respiratory infections were treated with antibiotics, 57% were treated with broad spectrum antibiotics. The proportion of women receiving antibiotic therapy for URIs, including broad spectrum antibiotics, was similar to adults in outpatient settings.[10, 83] In our study, URIs for which antibiotics are rarely indicated[79] accounted for 21% of total antibiotic use and 40% of broad spectrum antibiotic use. White women were more likely to receive an antibiotic for bronchitis, rhinosinusits, and nonspecific URI, and the use increased with increasing age. These

findings are consistent with studies evaluating determinants of receiving a broad-spectrum versus a narrow-spectrum antibiotic for URI in adults.[10, 84]

After removing antibiotic use for treatment upper respiratory infections, predictors of antibiotic use were similar to the known risk factors for genital tract infections, including numbers of sexual partners, black race, and the absence of predominant vaginal lactobacilli.[22, 85, 86] A novel feature of our study was the evaluation of correlates for appropriate antibiotic use in non-pregnant women.

Major strengths of our study include the prospective longitudinal design and the large sample of 650 women with over 5500 visits. However, there were a few limitations. Antibiotic exposure in the last 2 months was ascertained by interview which is subject to recall error. However, a recent study reported 89% agreement between self-reported antibiotic use in the previous 3 months and pharmacy database records.[87] The indications for antibiotic use were also identified through participant reports; therefore the diagnoses listed could not be confirmed. Information on whether diagnostic tests were performed was also lacking. Thus, it was likely that some cases of urinary tract infections and pharyngitis may not have been due to bacterial infections as was assumed for the analyses in this study. Therefore, the amount of inappropriate antibiotic use may have been underestimated. While this study identified participant factors that were independently associated with antibiotic use, no information on the types of providers who provided the prescriptions was available. Many studies have found that health provider characteristics, such as age, specialty, and practice type influence antibiotic prescribing.[10, 84, 88] Finally, this was a young, sexually active population which limits generalizability of results; results may not be applicable to pregnant or older women.

Antibiotic use is widespread among reproductive-aged, non-pregnant women. While the majority of the antibiotic use was appropriate given that the primary indications were genitourinary infections, broad-spectrum agents were often selected to treat URI conditions for which antibiotics provide little therapeutic benefit. Since little is known about the impact of antimicrobial use on selection for antibiotic resistant strains and adversely affecting the healthy microbiome, educational efforts to promote judicious use of antibiotics tailored to non-pregnant women and their healthcare providers would have broad public health benefit. Furthermore, preventing acquisition of sexually transmitted infections would have the cross benefit of reducing overall antibiotic exposure in women.

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4.7 TABLES

Table 4.1 Antibiotic use by class

Antibiotic Class	No. of Prescriptions Reported	Antibiotic Use Rate* (95% CI)
Total prescriptions	699	81.2 (75.4-87.5)
Metronidazole	169 (24.2%)	19.6 (16.9-22.8)
Penicillins	158 (22.6%)	18.4 (15.7-21.5)
Macrolides	106 (15.2%)	12.3 (10.2-14.9)
Fluoroquinolones	61 (8.7%)	7.1 (5.5-9.1)
Cephalosporins	57 (8.2%)	6.6 (5.1-8.6)
Sulfonamides	47 (6.7%)	5.5 (4.1-7.3)
Tetracyclines	42 (6.0%)	4.9 (3.6-6.6)
Nitrofurantoin	18 (2.6%)	2.1 (1.3-3.3)
Lincosamides	10 (1.4%)	1.2 (0.6-2.2)
Aminoglycosides	1 (0.1%)	0.1 (0.02-0.8)
Unknown	30 (4.3%)	3.5 (2.4-5.0)

* Number of antibiotic prescriptions per 100 person-years

Table 4.2 Antibiotic use by class and indication

Category	No. of Prescriptions (N=699)	MTZ N=169	PCN N=158	MAC N=106	QUIN N=61	CEPH N=57	Other* N=148
Genitourinary infection	339 (48.5%)	163 (96.4%)	7 (4.4%)	30 (28.3%)	38 (62.3%)	22 (38.6%)	79 (53.4%)
Bacterial vaginosis	144 (20.6%)†	143 (84.6%)†	0	0	0	0	1 (0.7%)
Urinary tract infection	93 (13.3%)	0	3 (1.9%)	2 (1.9%)	26 (42.6%)	1 (1.7%)	61 (41.2%)
Sexually transmitted infection	76 (10.9%)†	20 (11.8%)†	0	27 (25.5%)	8 (13.1%)	16 (28.1%)	5 (3.4%)
Pelvic inflammatory disease	11 (1.6%)	4 (2.4%)	2 (1.3%)	1 (0.9%)	1 (1.6%)	1 (1.7%)	2 (1.4%)
Other	19 (2.7%)	0	2 (1.3%)	0	3 (4.9%)	4 (7.0%)	10 (6.8%)
Upper respiratory infection	183 (26.2%)	0	81 (51.3%)	63 (59.4%)	14 (23.0%)	8 (14.0%)	17 (11.5%)
Pharyngitis	30 (4.3%)	0	20 (12.7%)	6 (5.7%)	0	2 (3.5%)	2 (1.4%)
Pneumonia	5 (0.7%)	0	0	3 (2.8%)	1 (1.6%)	1 (1.7%)	0
Bronchitis	28 (4.0%)	0	6 (3.8%)	15 (14.2%)	4 (6.6%)	0	3 (2.0%)
Rhinosinusitis	69 (9.9%)	0	37 (23.4%)	19 (17.9%)	6 (9.8%)	1 (1.7%)	6 (4.1%)
Non-specific‡	51 (7.3%)	0	18 (11.4%)	20 (18.9%)	3 (4.9%)	4 (7.0%)	6 (4.1%)
Dental indications	67 (9.6%)	0	52 (32.9%)	5 (4.7%)	0	1 (1.7%)	9 (6.1%)
Dental cleaning prophylaxis	6 (0.9%)	0	6 (3.8%)	0	0	0	0
Dental infection	19 (2.7%)	0	14 (8.9%)	2 (1.9%)	0	1 (1.7%)	2 (1.4%)
Tooth extraction/surgery	22 (3.1%)	0	17 (10.8%)	2 (1.9%)	0	0	3 (2.0%)
Tooth or gum abscess	14 (2.0%)	0	10 (6.3%)	1 (0.9%)	0	0	3 (2.0%)
Tooth pain	6 (0.9%)	0	5 (3.2%)	0	0	0	1 (0.7%)
Skin conditions	56 (8.0%)	0	5 (3.2%)	2 (1.9%)	1 (1.6%)	17 (29.8%)	31 (20.9%)
Abscess/cellulitis	12 (1.7%)	0	2 (1.3%)	0	0	9 (15.8%)	1 (0.7%)
Acne	19 (2.7%)	0	0	1 (0.9%)	0	1 (1.7%)	17 (11.5%)
Dermatitis/rash	8 (1.1%)	0	2 (1.3%)	0	0	3 (5.3%)	3 (2.0%)
Infection	9 (1.3%)	0	0	0	1 (1.6%)	2 (3.5%)	6 (4.1%)
Wound	8 (1.1%)	0	1 (0.6%)	1 (0.9%)	0	2 (3.5%)	4 (2.7%)
Other indications§	54 (7.7%)	6 (3.6%)	14 (8.9%)	6 (5.7%)	9 (14.8%)	8 (14.0%)	12 (8.1%)
Gastrointestinal indications	22 (3.1%)	6 (3.6%)	0	2 (1.9%)	7 (11.5%)	2 (3.5%)	5 (3.4%)
Ear infection	15 (2.1%)	0	11 (7.0%)	3 (2.8%)	1 (1.6%)	0	0
Eye infection	3 (0.4%)	0	0	1 (0.9%)	1 (1.6%)	0	1 (0.7%)
Musculoskeletal indications	4 (0.6%)	0	1 (0.6%)	0	0	3 (5.3%)	0
Miscellaneous indications	10 (1.4%)	0	1 (0.6%)	0	0	3 (5.3%)	6 (4.1%)

MTZ, metronidazole; PCN, penicillins; MAC, macrolides; QUIN, fluoroquinolones; CEPH, cephalosporins

*****Other antibiotics included tetracyclines, sulfonamides, nitrofurantoin, lincosamides, aminoglycosides, and unknown.

† 4 women had concomitant diagnoses of bacterial vaginosis and *Trichomonas vaginalis* and treated with metronidazole.

‡ Non-specific upper respiratory infection included cold, cough, flu, laryngitis, nasal infection, sore throat, tonsillitis, upper respiratory infection.

§ Gastrointestinal indications included appendicitis (n=3), diarrhea (n=2), gastroenteritis (n=2), *H. pylori* infection (n=2), surgery prophylaxis (n=3), stomach virus (n=1), typhoid fever (n=3), inflamed small intestine (n=2), abdominal abscess (n=2), and food infection prophylaxis (n=2); musculoskeletal indications included fracture (n=2), sprained wrist (n=1), and motor vehicle accident (n=1); miscellaneous indications included unspecified infection prophylaxis (n=2), Lyme disease (n=1), malaria prophylaxis (n=1), mastitis (n=2), surgery prophylaxis (n=2), and swollen lymph glands (n=2).

Table 4.3 Independent risk factors for receiving antibiotics for bronchitis, rhinosinusitus, or non-specific upper respiratory tract infection (N=229 women)

Variable	No. URI events* (N=340)	No (%) Treated with Antibiotic (n=143)	Unadjusted RR (95% CI)	Adjusted* RR (95% CI)
Site				
Pittsburgh, PA	251	104 (41.4)	Referent	
Augusta, GA	64	34 (53.1)	1.27 (0.94-1.72)	
Houston, TX	25	5 (20.0)	0.41 (0.11-0.81)	
Race				
White	235	110 (46.8)	1.47 (1.03-2.08)	1.51 (1.08-2.10)
Non-white	105	33 (31.4)	Referent	Referent
Age, years				
18-19	24	7 (29.2)	Referent	Referent
20-24	142	51 (35.9)	1.33 (0.59-2.96)	1.35 (0.62-2.94)
25-29	104	46 (44.2)	1.66 (0.74-3.70)	1.66 (0.76-3.63)
30-34	44	23 (52.3)	1.84 (0.79-4.28)	1.88 (0.83-4.24)
35-40	26	16 (61.5)	2.29 (0.99-5.29)	2.42 (1.05-5.57)
Education, years				
8-12	86	38 (44.2)	Referent	
13-16	198	84 (42.4)	1.01 (0.73-1.41)	
17 or more	56	21 (37.5)	0.84 (0.52-1.36)	

URI, bronchitis, rhinosinusitus, or non-specific upper respiratory tract infection; RR, relative risk; CI, confidence interval
*Adjusted for race and age.

Table 4.4 Antibiotic use rates by demographic and behavioral characteristics (N=699 prescriptions)

Variable	No. of Prescriptions	Woman-years	Antibiotic Use Rate*	Unadjusted IRR (95% CI)
Site				
Pittsburgh, PA	551	593.7	92.8	Referent
Augusta, GA	116	234.3	49.5	0.53 (0.42-0.68)
Houston, TX	32	32.8	97.6	1.01 (0.69-1.47)
Vaccine arm				
Tetanus-diphtheria	362	438.1	82.6	Referent
GBS type III	337	422.8	79.7	0.96 (0.79-1.18)
Race				
White	386	523.2	68.0	Referent
Black	264	279.9	94.3	1.27 (1.03-1.58)
Other	49	57.8	84.8	1.15 (0.77-1.74)
Age, years				
18-19	51	87.4	58.4	Referent
20-24	299	359.1	83.1	1.35 (0.91-2.00)
25-29	207	221.8	93.3	1.55 (1.04-2.31)
30-34	78	93.8	83.2	1.36 (0.84-2.19)
35-40	61	98.3	62.1	1.00 (0.61-1.66)
Education, years				
8-12	216	215.3	100.3	1.64 (1.21-2.23)
13-16	364	450.1	80.9	1.33 (1.02-1.74)
17 or more	119	195.4	60.9	Referent
Employed				
No	233	250.0	93.2	Referent
Yes	466	610.8	76.3	0.83 (0.68-1.02)
Marital status				
Single	586	693.4	84.5	Referent
Married	113	167.4	67.5	0.83 (0.63-1.07)
Contraception since last visit†				
None, spermicide, tubal ligation, or copper intrauterine device	124	174.1	71.2	Referent
Barrier methods	151	180.4	83.7	1.18 (0.87-1.60)
Hormonal methods, not DMPA	352	416.1	84.6	1.28 (0.98-1.68)
DMPA	72	90.3	79.7	1.12 (0.75-1.65)
New sex partner since last visit				
No	628	798.0	78.7	Referent
Yes	71	62.9	112.9	1.38 (1.02-1.88)
Number of sex partners since last visit				
None	69	103.8	66.5	Referent
One	589	723.3	81.4	1.27 (0.95-1.71)
Two or more	41	33.7	121.7	1.67 (1.05-2.67)
Nugent score at prior visit‡				
Normal (0-3)	350	510.1	68.6	Referent
Intermediate (4-6)	127	129.4	98.1	1.43 (1.14-1.79)
Bacterial vaginosis (7-10)	222	214.1	103.7	1.57 (1.28-1.93)

IRR, incidence rate ratio; CI, confidence interval, DMPA, depot medroxyprogesterone acetate

* Prescribing rate is per 100 person-years.

† Barrier methods (condoms, diaphragms, cervical caps); hormonal methods, not DMPA (oral contraceptive tablets, vaginal rings, intrauterine devices containing levonorgestrel, transdermal patches, implants).

‡ Nugent score missing for 9 visits

Table 4.5 Independent risk factors for antibiotic use, excluding use for upper respiratory tract infections (N=516 prescriptions)

Variable	Adjusted* IRR (95% CI)	*P*-value
Site		<0.001
Pittsburgh, PA	Referent	
Augusta, GA	0.49 (0.36-0.66)	
Houston, TX	0.87 (0.56-1.36)	
Race		0.004
White	Referent	
Black	1.48 (1.15-1.90)	
Other	1.18 (0.69-2.01)	
Age, years		0.001
18-19	Referent	
20-24	1.29 (0.80-2.09)	
25-29	1.29 (0.78-2.13)	
30-34	0.94 (0.52-1.70)	
35-40	0.73 (0.40-1.35)	
Number of sex partners since last visit		<0.001
None	Referent	
One	1.48 (1.03-2.14)	
Two or more	2.04 (1.19-3.50)	
Abnormal Nugent score at prior visit (4-10)	1.80 (1.45-2.24)	<0.001

IRR, incidence rate ratio; CI, confidence interval;
*Adjusted for study site, race, age, number of sex partners, and Nugent score

5.0 MANUSCRIPT 2: ASSOCIATION OF CLASS-SPECIFIC ANTIBIOTIC USE WITH VAGINAL AND RECTAL COLONIZATION BY *LACTOBACILLUS*

To be submitted for publication

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5.1 ABSTRACT

Objective: To evaluate the association of class-specific antibiotic use with vaginal and rectal *Lactobacillus* colonization among non-pregnant women.

Study Design: 650 asymptomatic women were followed bi-monthly for up to 18 months in a randomized vaccine trial. Antibiotic use and vaginal and rectal swabs for culture detection of lactobacilli were obtained at each visit. Generalized estimating equations and multinomial logistic regression were used to evaluate risk factors for vaginal and rectal colonization by lactobacilli.

Results: The self-reported antibiotic use rate was 81.2 per 100 woman-years. Women were colonized by hydrogen peroxide (H_2O_2)-producing lactobacilli in the vagina and rectum at 3941 (70.4%) and 3514 (62.8%) of 5597 follow-up visits, respectively. After adjusting for vaccine arm, race, age, education, and contraceptive method, women who used β-lactam antibiotics in the previous 14 days had a 45% decreased odds of vaginal colonization by H_2O_2-producing lactobacilli (95% confidence interval (CI): 0.38-0.78) than women who denied antibiotic exposure. β-lactam use was associated with a ~34% decreased odds of rectal colonization, irrespective of time since exposure, after adjustment for vaccine arm, age, and race. For the three predominant H_2O_2-producing species, β-lactam use was associated with decreased vaginal *L. jensenii* and *L. gasseri* colonization, and decreased rectal colonization by *L. crispatus* and *L. gasseri*. Use of other classes of antibiotics did not significantly impact colonization by H_2O_2-producing lactobacilli.

Conclusion: In this population of women having a high antibiotic use burden, the use of β-lactam antibiotics was associated with reduced colonization by H_2O_2-producing lactobacilli in both the vagina and rectum.

5.2 INTRODUCTION

Although antibiotics are one of the most commonly prescribed classes of medications, it is estimated that about half of antibiotic use is either unnecessary or sub-optimally effective as prescribed.[3] Widespread antibiotic use in humans and in commercial agriculture has contributed to the emergence of antibiotic resistant bacteria. The Centers for Disease Control and Prevention recently classified antibiotic resistant bacteria according to their potential impact on public health and drug resistant *Neisseria gonorrhoeae* was assigned the highest threat level of urgent. In 2011, 246,000 *N. gonorrhoeae* isolates, representing 30% of cases of gonococcal infections in the US, were resistant to at least one antibiotic commonly used for treatment. If not controlled, the spread of drug resistant *N. gonorrhoeae* infections could result in an estimated additional 75,000 cases of pelvic inflammatory disease, a major cause of infertility, and 222 human immunodeficiency viral (HIV) infections over the next 10 years.[3]

In addition to contributing to an increase in multidrug resistant pathogens, antibiotic use also has a deleterious impact on commensal bacteria which colonize the gastrointestinal, respiratory and reproductive tracts. *Lactobacillus* species are the predominant bacteria in the healthy vaginal microbiome, and *Lactobacillus crispatus* have been shown to be an important predictor of a stable vaginal microbiome.[15] Women who harbor hydrogen peroxide–producing lactobacilli in the vagina and/or the rectum have a reduced incidence of bacterial vaginosis, which is the most common vaginal syndrome among reproductive-age women.[16, 17] Bacterial vaginosis is characterized by the absence of lactobacilli and the overgrowth of *Gardnerella vaginalis* and a diverse group of anaerobic bacteria, and is associated with increased acquisition of sexually transmitted infections including *N. gonorrhoeae,* HIV, herpes simplex virus type 2, *Chlamydia trachomatis,* and *Trichomonas vaginalis*.[18-22]

It is unclear whether antibiotics being used in women have a deleterious impact on vaginal lactobacilli and thereby increase their risk for acquiring vaginal infections. There have been few studies which evaluated the effect of antimicrobial use on vaginal colonization by *Lactobacillus* beyond those used for the treatment of bacterial vaginosis.[23, 24] Recent self-reported antibiotic use was associated with reduced vaginal *Lactobacillus* colonization in two prospective cohort studies.[14, 25] However, susceptibility patterns vary by antibiotic class and studies that have prospectively assessed the effect of specific antibiotics on vaginal *Lactobacillus* colonization have been limited by small sample sizes.[26-32] Furthermore, the association between antibiotic use and rectal *Lactobacillus* colonization has not been evaluated. Since the rectum is considered a reservoir for many species of bacteria which colonize the vagina, it would be important to determine whether antibiotic use affects *Lactobacillus* colonization at this site. The objective of this longitudinal study was to evaluate the association of class-specific antibiotic use with vaginal and rectal *Lactobacillus* colonization among non-pregnant women.

5.3 METHODS

5.3.1 Study population

This is a secondary analysis of a randomized, clinical trial designed to evaluate the efficacy of a group B *Streptococcus* (GBS) serotype III polysaccharide-tetanus toxoid conjugated vaccine. Briefly, non-pregnant, sexually active women aged 18-40 years who were using effective birth control methods, denied antimicrobial and antifungal use in the past 7 days, and were GBS culture-negative both vaginally and rectally were recruited from primary care and family planning settings in Pittsburgh, PA, Augusta, GA, and Houston, TX. Participants (n=663) were

randomized to receive a single dose of GBS type III- tetanus toxoid vaccine or a licensed vaccine containing tetanus and diphtheria toxoids adsorbed for adult use. The clinical trial was approved by the institutional review boards of the participating institutions. All participants provided written informed consent. The trial enrollment occurred from July 2003 to August 2006, and all follow-up visits were completed by February 2008.

5.3.2 Antibiotic use and *Lactobacillus* identification

Vaginal and rectal swabs for culture detection of lactobacilli and demographics, behavioral information, and use of systemic or intra-vaginal antimicrobials, were collected by pelvic examination and interview at enrollment, 1- and 2-months, and then bi-monthly for 18 months. Antimicrobial use was categorized by antibiotic class and by mechanism of action as follows: β-lactams (penicillins and cephalosporins); protein synthesis inhibitors (macrolides, lincosamides, tetracyclines); nucleic acid synthesis inhibitors (fluoroquinolones, nitrofurantoin); and others (sulfonamides, class not determined). *Lactobacillus* were isolated and identified by colony and Gram stain morphology. Hydrogen peroxide (H_2O_2) detection of the *Lactobacillus spp* isolated was performed by a qualitative method in which isolates are inoculated onto a brucella agar base supplemented with 3,3',5,5'-tetramethylbenzidine and horseradish peroxidase. Anaerobic incubation at 37°C for 48 to 72 hours followed by exposure to air for 30 minutes results in a blue pigment if there is H_2O_2 production and no pigment if H_2O_2 production is absent.[89] The lactobacilli isolated from women in the tetanus-diphtheria vaccine arm were identified to the species level using repetitive sequence polymerase chain reaction fingerprinting or 16S-rDNA PCR for restriction fragment length polymorphism (RFLP) analysis using HaeIII (Promega,

Madison, WI) and HpyCH4V (New England Biolab, Ipswich, MA) restriction enzymes. The RFLP patterns for each species were confirmed by sequencing the 16S rDNA.[90]

5.3.3 Covariates

Variables considered as potential explanatory factors included study site, vaccine arm, race, age, education, and marital and employment status at enrollment. Other variables which could change over time and for which data was collected at each visit included number of male sexual partners, new sex partner, frequency of vaginal intercourse, douching, antibiotic use, and contraceptive methods. Contraceptives were categorized as: none, non-barrier methods (spermicides, tubal ligations, or copper intrauterine devices), barrier methods (condoms, diaphragms, or cervical caps), hormonal methods (oral contraceptive tablets, intrauterine devices containing levonorgestrel, the vaginal ring, transdermal patches, or implants), and depot medroxyprogesterone acetate (DMPA).

5.3.4 Statistical analysis

Statistical analyses were performed using Stata statistical software release 11.2 (Stata Corp., College Station, TX), and statistical tests were evaluated at the 0.05 two-sided significance level. All women who completed at least one follow-up visit (N=650) were included in the analyses. A population averaged generalized estimating equations regression model based on the repeated visit measures per woman was used to evaluate risk factors for vaginal and rectal colonization by H_2O_2-producing *Lactobacillus* and estimate the population averaged odds ratios (OR). An exchangeable working correlation matrix was specified and modified sandwich estimates of the

variance were calculated.[78] Variables were retained in the model if the *P* value from the Wald chi-squared test statistic was 0.05 or less. The final models were also adjusted for age. Generalized estimating equations regression was also used to evaluate the association between antibiotic use and vaginal colonization by *L. iners*. Multinomial logistic regression was used to evaluate the association between antibiotic use and vaginal and rectal colonization by *L. crispatus*, *L. jensenii*, and *L. gasseri*. Because women can be concurrently colonized by multiple species of lactobacilli, the *Lactobacillus* species-specific colonization outcome was defined as follows: absence of *L. crispatus*, *L. jensenii*, and *L .gasseri* (the reference category); presence of *L. crispatus*; presence of *L. jensenii* in the absence of *L. crispatus*; and presence of *L. gasseri* in the absence of both *L. crispatus* and *L. jensenii*. The 95% confidence intervals were based on the robust estimator of the standard errors which accounted for the repeated measures per woman.

5.4 RESULTS

This analysis included 650 women who completed 5599 follow-up visits over 860.8 woman-years. Most women (60%) completed all 10 scheduled follow-up visits and 91% completed 5 or more visits; the median follow-up time was 1.5 years. There were no differences in demographic characteristics between women who completed all study visits and those that did not with the exception of age. Women who completed all scheduled visits were slightly older than those with fewer than 10 visits (26.6$\pm$5.8 years and 24.4$\pm$5.2 years, respectively, $p<0.001$). Antimicrobial use was reported by 341 (52.5%) women at least once and at 605 (10.8%) of the follow-up visits. Women reported using a total of 699 antibiotic prescriptions for an overall antibiotic use rate of 81.2 per 100 woman-years of follow-up (95% confidence interval (CI): 75.4-87.5). The use of more than one antibiotic was reported at 75 visits; 2 at 52 visits, 3 at 21 visits, and 4 at 2 visits.

The most commonly reported antibiotics used were β-lactams (30.8%), metronidazole (24.2%), and protein synthesis inhibitors (22.6%, Table 5.1). The majority of antibiotics were used to treat genitourinary conditions (48.5%); primarily bacterial vaginosis (20.6%), urinary tract infections (13.3%), and sexually transmitted infections (10.9%). However, upper respiratory tract infections accounted for 26.2% of all antibiotic use and 41.4% of the use of β-lactam agents.

Vaginal and rectal swab specimens for *Lactobacillus* culture were not evaluable for two visits, so the following analyses included 5597 visits. Women were colonized by H_2O_2-producing lactobacilli in the vagina at 3941 (70.4%) and in the rectum at 3514 (62.7%) of the follow-up visits. The univariate associations between demographics and contraceptive methods with vaginal and rectal colonization by H_2O_2-producing *Lactobacillus* are shown in Table 5.2.

Non-white race and having less than 17 years of education were associated with reduced colonization by H_2O_2-producing lactobacilli at either anatomical site. Women who used hormonal contraception methods, not including DMPA, were more likely to be vaginally or rectally colonized by H_2O_2-producing lactobacilli than women who used no contraception, spermicides, or copper intrauterine devices and those who had tubal ligations. DMPA use was not significantly associated with *Lactobacillus* colonization. Women who received the GBS type III vaccine were more likely to be colonized by H_2O_2-producing lactobacilli in the rectum than women who received the tetanus-diphtheria vaccine (Table 5.2). Age, marital and employment status, douching in the past 30 days, and sexual activity were not associated with *Lactobacillus* colonization ($p>0.05$).

While overall antibiotic use was not associated with *Lactobacillus* colonization, women who used β-lactams had a 26% decreased odds of colonization by H_2O_2-producing lactobacilli in the vagina (95% CI: 0.58-0.95) and a 34% decreased odds of colonization in the rectum (95%

CI: 0.51-0.85) than women who denied antibiotic exposure since their last visit in the unadjusted analyses (Table 5.3). Other classes of antibiotics were not significantly associated with H_2O_2-producing *Lactobacillus* colonization. After taking into account time since last dose, more recent exposure to β-lactams (within 14 days) was associated with a 44% decrease in the odds of colonization by H_2O_2-producing lactobacilli in the vagina, while exposure >14 days was not associated (odds ratio (OR)=0.94). Time since exposure to β-lactams did not alter the magnitude of the association of β-lactam antibiotics with rectal *Lactobacillus* colonization (Table 5.3), even when time since last dose was categorized by 10 day intervals up to 30 days and then by >30 days (data not shown).

Antibiotic use, race, education, and contraceptive methods were independently associated with vaginal colonization by H_2O_2-producing lactobacilli (Table 5.4). The use of β-lactam antibiotics in the previous 14 days remained associated with reduced colonization by H_2O_2-producing lactobacilli in the vagina (adjusted odds ratio (aOR) =0.55, 95% CI: 0.38-0.78) compared to no antibiotic exposure. Other classes of antibiotics and β-lactam use >14 days were not significantly associated with decreased vaginal *Lactobacillus* colonization. Women with 8-12 years and 13-16 years of education were less likely to be colonized by H_2O_2-producing lactobacilli in the vagina compared to women with 17 years or more (aOR=0.46, 95% CI: 0.30-0.69 and aOR=0.66, 95% CI: 0.46-0.96, respectively), while hormonal methods of contraception, excluding DMPA, was associated with an increased likelihood of colonization (aOR=1.31, 95% CI: 1.03-1.67) compared to non-barrier methods or no contraception. Black women were less likely than white women to be colonized at either site (vaginal adjusted odds ratio (aOR)=0.51, 95% CI: 0.39-0.66 and rectal aOR=0.63, 95% CI: 0.51-0.78).

In addition to race, antibiotic use and vaccine arm were the factors independently associated with rectal colonization by H_2O_2-producing lactobacilli (Table 5.4). β-lactam antibiotic use, irrespective of time since last dose, was associated with a ~34% decrease in the odds of rectal colonization compared to women with no antibiotic exposure (≤14 days aOR=0.65, 95% CI: 0.47-0.90 and >14 days aOR=0.66, 95% CI: 0.45-0.97). Women who received the GBS type III vaccine were more likely to be colonized by H_2O_2-producing lactobacilli in the rectum than women who received the tetanus-diphtheria vaccine (aOR=1.22, 95% CI: 1.01-1.49).

To assess whether the antibiotic effects were specific to particular species of lactobacilli, the isolates were identified to the species level for the 330 women in the tetanus-diphtheria vaccine arm for a total of 2855 visits. The species-specific prevalence of vaginal and rectal colonization was: *L. crispatus* (39.4% and 33.6%); *L. jensenii* (28.0% and 13.2%); *L. gasseri* (22.3% and 26.2%); and *L. iners* (24.6% and 1.2%). While β-lactam use was not associated with a statistically significant decrease in vaginal colonization by *L.* crispatus, rectal colonization by *L. crispatus* was associated with β-lactam use >14 days (OR=0.51, 95% CI: 0.27-0.99, Table 5.5). There was a 72% decrease in the odds of vaginal colonization by *L. jensenii* in the absence of *L. crispatus* colonization among women who used β-lactams in the last 14 days (95% CI: 0.08-0.93) compared to women with no antibiotic exposure. While there was a similar decrease in rectal colonization by *L. jensenii* among women reporting recent β-lactam use, this association was not statistically significant. In women whose predominant *Lactobacillus* species was *L. gasseri*, recent β-lactam use was associated with ~80% decrease in the odds of colonization by *L. gasseri* in both the vagina and the rectum. Although β-lactam use did not alter colonization by *L iners*, use of protein synthesis inhibitors was associated with a 52% decreased odds of vaginal

colonization by this microorganism (95% CI: 0.25-0.90) compared to no antibiotic use (data not shown).

5.5 DISCUSSION

In this large prospective study of reproductive-aged women, the use of β-lactam antibiotics was associated with a 34 to 45% reduction in the odds of colonization by H_2O_2-producing lactobacilli in both the vagina and rectum, while other classes of antibiotics or overall self-reported antibiotic use were not associated with *Lactobacillus* colonization. Self-reported antibiotic use has been reported as a risk factor for loss of H_2O_2-producing lactobacilli in the vagina in two previous studies, and the magnitude of the association was similar in a Kenyan study of over 1000 sex workers.[14, 25] However, both of these studies evaluated measures of overall antibiotic use and did not assess class-specific associations. Among three studies that did prospectively evaluate the use of penicillins in women without bacterial vaginosis, one found decreased colonization by vaginal lactobacilli one-week after discontinuation of therapy, while the other two studies found no change in vaginal *Lactobacillus* colonization.[27-29] However, these three studies evaluated a combined total of 41 women.

The association between β-lactam use and rectal colonization by H_2O_2-producing lactobacilli did not vary by time since exposure, whereas only more recent use was associated with vaginal colonization in this study. These results suggest that the disruption of *Lactobacillus* colonization associated with the use of β-lactam antibiotics persists in the rectum for a longer period of time than in the vagina. Previous studies of perturbation of the distal gut microbiome following use of a fluoroquinolone have demonstrated that there is an incomplete rebound in the microbiota that persists for 2 to 6 months after discontinuation of antibiotic therapy.[91]

The primary *Lactobacillus* species that produce hydrogen peroxide in the vagina are *L. crispatus, L.jensenii,* and *L. gasseri*.[72] A recent study that assessed the susceptibility of vaginal and rectal *Lactobacillus* isolates to ampicillin, found that 80% of *L. crispatus* isolates, 87% of *L. jensenii*, and all *L. gasseri* isolates were susceptible to this β-lactam antibiotic *in vitro*.[68] In the present study, while the use of β-lactam antibiotics was associated with reduced colonization by any H_2O_2-producing lactobacilli, species-specific analyses showed that the use of β-lactams was associated with decreased colonization by *L. crispatus* in the rectum but not in the vagina. Therefore, although 80% of *L. crispatus* isolates are susceptible to β-lactams *in vitro*, the use of β-lactams did not appear to have a sustained inhibitory effect on *L. crispatus in vivo*. This is an important finding since women who are colonized by *L. crispatus* have a reduced incidence of bacterial vaginosis, and thereby have a decreased risk of acquiring other vaginal infections.[73, 74] However, β-lactam use in the previous two weeks was associated with marked decreases in vaginal and rectal *Lactobacillus* colonization in women whose predominant H_2O_2-producing *Lactobacillus* species were *L. jensenii or L. gasseri*, which is consistent with their susceptibility to β-lactams. Most *L. iners* isolates do not produce hydrogen peroxide.[72] Unlike the predominantly H_2O_2-producing *Lactobacillus* species evaluated in this study, β-lactam use was not associated with decreased vaginal colonization by *L. iners* even though this species is susceptible to β-lactam antibiotics. However, the relative prevalence of *L. iners* was low, thus limiting the statistical power to detect changes in colonization following antibiotic therapy. *L. iners* colonization was decreased among women who used macrolides, lincosamides, or tetracyclines.

To date, this is the first study to assess the association of antibiotic use with rectal colonization by H_2O_2-producing lactobacilli. Women colonized by H_2O_2-producing lactobacilli

in both the vagina and rectum have a lower prevalence of bacterial vaginosis than women colonized at only one of these sites, suggesting that the rectum is a reservoir for lactobacilli which colonize the vagina.[17] Therefore, the finding that the use of β-lactam antibiotics is associated with reduced rectal as well as vaginal colonization by lactobacilli may have important public health implications in that women using these antimicrobial agents may be at increased risk for acquiring bacterial vaginosis. Since β-lactam antibiotics were often selected to treat upper respiratory tract infections for which antibiotics typically provide little therapeutic benefit, women and their healthcare providers should be aware that the inappropriate use of antibiotics may disrupt the normal microbiota and increase susceptibility to other infections.

Black women were less likely to be colonized by H_2O_2-producing lactobacilli in the vagina and the rectum, and women with an education attainment equivalent to less than a college graduate were less likely to be vaginally colonized in this study. This was not surprising since race and low educational attainment have been identified as correlates of vaginal *Lactobacillus* colonization and/or bacterial vaginosis in other studies.[72, 92] Contraceptive methods that contained hormones, which included oral contraceptives, hormonal intrauterine devices, vaginal rings, transdermal patches, and implants was associated with an increased likelihood of vaginal colonization by H_2O_2-producing lactobacilli in this study. DMPA differs from other hormonal contraceptives in that it has an anti-estrogenic effect on the vaginal epithelium, and this contraceptive method was not correlated with colonization by lactobacilli in the present study. Since there is an inverse relationship between *Lactobacillus* colonization and bacterial vaginosis, these findings are consistent with a recent meta-analysis of 55 studies, which found that hormonal contraceptive use, regardless of form, was associated with a reduced risk of bacterial vaginosis.[93]

Major strengths of our study include the prospective longitudinal design, the large sample of 650 women with 5600 visits, and assessment of both vaginal and rectal *Lactobacillus* colonization by culture methods, and identification of the lactobacilli to the species level using 16S sequencing based techniques. A limitation of this study was that antibiotic exposure in the last 2 months was ascertained during a structured interview, a method which is subject to recall error. However, a recent study reported 89% agreement between self-reported antibiotic use in the previous 3 months and pharmacy database records.[87] Finally, this was a young, sexually active population which limits generalizability of results.

In this population of reproductive-aged women having a high antibiotic use burden, the use of β-lactam antibiotics was associated with reduced colonization by hydrogen peroxide-producing lactobacilli in both the vagina and rectum. Since the use of some classes of antibiotics adversely affect the healthy vaginal microbiome, educational efforts to promote appropriate use of antibiotics tailored to non-pregnant women and their healthcare providers would have broad public health benefit.

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5.7 TABLES

Table 5.1 Antibiotic use by class

Antibiotic Class	No. of Prescriptions Reported
Total prescriptions	699
β-lactams	215 (30.8%)
Penicillins	158 (22.6%)
Cephalosporins	57 (8.2%)
Metronidazole	169 (24.2%)
Protein synthesis inhibitors	158 (22.6%)
Macrolides	106 (15.2%)
Tetracyclines	42 (6.0%)
Lincosamides	10 (1.4%)
Nucleic acid synthesis inhibitors	79 (11.3%)
Fluoroquinolones	61 (8.7%)
Nitrofurantoin	18 (2.6%)
Others	78 (11.2%)
Sulfonamides	47 (6.7%)
Aminoglycosides	1 (0.1%)
Unknown	30 (4.3%)

Table 5.2 Association of demographics and contraceptive methods with vaginal and rectal colonization by hydrogen peroxide-producing *Lactobacillus*

Variable	No. Total Visits (N=5597)	Vaginal Colonization by H_2O_2-producing *Lactobacillus*		Rectal Colonization by H_2O_2-producing *Lactobacillus*	
		No. (%) Visits Present (n=3941)	Unadjusted OR (95% CI)	No. (%) Visits Present (n=3514)	Unadjusted OR (95% CI)
Site					
Pittsburgh, PA	3929	2750 (70.0)	Referent	2449 (62.3)	Referent
Augusta, GA	1441	1073 (74.5)	1.19 (0.89-1.61)	932 (64.6)	1.09 (0.87-1.38)
Houston, TX	227	118 (52.0)	0.43 (0.25-0.76)	133 (58.6)	0.79 (0.52-1.21)
Vaccine arm					
Tetanus-diphtheria	2855	1943 (68.1)	Referent	1717 (60.1)	Referent
GBS type III	2742	1998 (72.9)	1.25 (0.98-1.59)	1797 (65.5)	1.25 (1.03-1.52)
Race					
White	3385	2617 (77.3)	Referent	2268 (67.0)	Referent
Black	1830	1079 (59.0)	0.42 (0.32-0.54)	1024 (56.0)	0.63 (0.51-0.77)
Other	382	245 (64.1)	0.58 (0.36-0.92)	222 (58.1)	0.73 (0.51-1.05)
Age, years					
18-19	549	394 (71.8)	Referent	358 (65.2)	Referent
20-24	2318	1707 (73.6)	1.03 (0.64-1.65)	1528 (65.9)	1.00 (0.69-1.44)
25-29	1458	989 (67.8)	0.79 (0.48-1.29)	877 (60.2)	0.77 (0.52-1.13)
30-34	625	410 (65.6)	0.70 (0.40-1.24)	340 (54.4)	0.60 (0.38-0.94)
35-40	647	441 (68.2)	0.82 (0.47-1.43)	411 (63.5)	0.90 (0.58-1.39)
Education, years					
17 or more	1268	1019 (80.4)	Referent	871 (68.7)	Referent
13-16	2924	2104 (72.0)	0.62 (0.43-0.89)	1841 (63.0)	0.77 (0.59-1.01)
8-12	1405	818 (58.2)	0.35 (0.24-0.51)	801 (57.0)	0.60 (0.45-0.80)
Contraception since last visit*					
None or non-barrier methods	1093	672 (61.5)	Referent	614 (56.2)	Referent
Barrier methods	1188	768 (64.6)	0.99 (0.81-1.22)	716 (60.3)	0.99 (0.81-1.22)
Hormonal methods, not DMPA	2704	2104 (77.8)	1.45 (1.15-1.82)	1807 (66.8)	1.24 (1.01-1.53)
DMPA	612	397 (64.9)	1.10 (0.73-1.64)	377 (61.6)	1.21 (0.90-1.62)

OR, odds ratio; CI, confidence interval; DMPA, depot medroxyprogesterone acetate

*Non-barrier methods (spermicide, tubal ligation, copper intrauterine device); barrier methods (condoms, diaphragms, cervical caps); hormonal methods, not DMPA (oral contraceptive tablets, vaginal rings, intrauterine devices containing levonorgestrel, transdermal patches, implants).

Table 5.3 Association of antibiotic use with vaginal and rectal colonization by hydrogen peroxide-producing *Lactobacillus*

Antibiotic use since last visit*	No. Total Visits (N=5597)	Vaginal Colonization by H_2O_2-producing *Lactobacillus*		Rectal Colonization by H_2O_2-producing *Lactobacillus*	
		No. (%) Visits Present (n=3941)	Unadjusted OR (95% CI)	No. (%) Visits Present (n=3514)	Unadjusted OR (95% CI)
None	4992	3518 (70.5)	Referent	3140 (62.9)	Referent
Any antibiotic	605	423 (69.9)	1.01 (0.85-1.19)	374 (61.8)	0.92 (0.78-1.08)
>14 days since last dose	386	272 (70.5)	1.02 (0.84-1.24)	237 (61.4)	0.90 (0.74-1.09)
≤14 days since last dose	219	151 (68.9)	0.98 (0.75-1.27)	137 (62.6)	0.95 (0.74-1.23)
Class-specific antibiotic use					
β-lactams	214	133 (62.1)	0.74 (0.58-0.95)	112 (52.3)	0.66 (0.51-0.85)
β-lactams, >14 days since last dose	121	84 (69.4)	0.94 (0.65-1.35)	64 (52.9)	0.65 (0.47-0.90)
β-lactams, ≤14 days since last dose	93	49 (52.7)	0.56 (0.40-0.78)	48 (51.6)	0.66 (0.45-0.97)
Protein synthesis inhibitors	130	104 (80.0)	1.33 (0.99-1.77)	96 (73.8)	1.19 (0.87-1.63)
Metronidazole	130	83 (63.8)	1.15 (0.77-1.71)	71 (54.6)	0.93 (0.64-1.34)
Nucleic acid synthesis inhibitors	68	53 (77.9)	1.02 (0.68-1.54)	52 (76.5)	1.54 (0.99-2.42)
Others	63	50 (79.4)	1.38 (0.88-2.15)	43 (68.3)	1.09 (0.65-1.84)

OR, odds ratio; CI, confidence interval

* β-lactams (penicillins, cephalosporins); protein synthesis inhibitors (macrolides, lincosamides, tetracyclines); nucleic acid synthesis inhibitors (fluoroquinolones, nitrofurantoin); others (sulfonamides, unknown)

Table 5.4 Independent risk factors for vaginal and rectal colonization by hydrogen peroxide-producing *Lactobacillus*

Factor	Vaginal Colonization by H_2O_2-producing *Lactobacillus* Adjusted* OR (95% CI)	Rectal Colonization by H_2O_2-producing *Lactobacillus* Adjusted* OR (95% CI)
GBS serotype III vaccine arm	1.20 (0.94-1.54)	1.22 (1.01-1.49)
Race		
White	Referent	Referent
Black	0.51 (0.39-0.66)	0.63 (0.51-0.78)
Other	0.74 (0.45-1.21)	0.77 (0.53-1.10)
Age, years		
18-19	Referent	Referent
20-24	0.91 (0.57-1.47)	1.03 (0.70-1.50)
25-29	0.68 (0.41-1.12)	0.80 (0.54-1.18)
30-34	0.70 (0.39-1.26)	0.63 (0.40-1.00)
35-40	1.01 (0.57-1.79)	1.01 (0.64-1.60)
Education, years		
17 or more	Referent	
13-16	0.66 (0.46-0.96)	
8-12	0.46 (0.30-0.69)	
Contraception since last visit†		
None or non-barrier methods	Referent	
Barrier methods	1.03 (0.84-1.27)	
Hormonal methods, not DMPA	1.31 (1.03-1.67)	
DMPA	1.26 (0.85-1.86)	
Antibiotic use since last visit‡		
None	Referent	Referent
Antibiotics other than β-lactams	1.23 (1.01-1.50)	1.13 (0.91-1.40)
β-lactams, >14 days since last dose	0.94 (0.64-1.39)	0.65 (0.47-0.90)
β-lactams, ≤14 days since last dose	0.55 (0.38-0.78)	0.66 (0.45-0.97)

OR, odds ratio; CI, confidence interval; DMPA, depot medroxyprogesterone acetate

* Vaginal colonization adjusted for vaccine arm, race, age, education and antibiotic use; rectal colonization adjusted for vaccine arm, race, age, and antibiotic use.

† Non-barrier methods (spermicide, tubal ligation, copper intrauterine device); barrier methods (condoms, diaphragms, cervical caps); hormonal methods, not DMPA (oral contraceptive tablets, vaginal rings, intrauterine devices containing levonorgestrel, transdermal patches, implants).

‡ β-lactams (penicillins, cephalosporins); all other antibiotics (macrolides, lincosamides, tetracyclines, fluoroquinolones, nitrofurantoin, sulfonamides, unknown).

Table 5.5 Vaginal and rectal colonization by *Lactobacillus spp.* and antibiotic use among women in the tetanus-diphtheria vaccine arm – multinomial logistic regression model (N=330 women)

Antibiotic use since last visit*	No. Total Visits	Vaginal Colonization by *Lactobacillus spp*		Rectal Colonization by *Lactobacillus spp.*	
		No. (%) Visits Present	Unadjusted OR (95% CI)	No. (%) Visits Present	Unadjusted OR (95% CI)
L. crispatus present	2855	1125 (39.4)		960 (33.6)	
None	2544	1004 (39.5)	Referent	865 (34.0)	Referent
Antibiotics other than β-lactams	199	80 (40.2)	1.18 (0.82-1.71)	65 (32.7)	1.05 (0.73-1.52)
β-lactams, >14 days since last dose	59	19 (32.2)	0.76 (0.43-1.36)	13 (22.0)	0.51 (0.27-0.99)
β-lactams, ≤14 days since last dose	53	22 (41.5)	0.71 (0.40-1.28)	17 (32.1)	0.65 (0.36-1.18)
L. jensenii present, *L. crispatus* absent	2855	390 (13.7)		219 (7.7)	
None	2544	350 (13.8)	Referent	194 (7.6)	Referent
Antibiotics other than β-lactams	199	31 (15.6)	1.31 (0.80-2.16)	18 (9.0)	1.30 (0.69-2.47)
β-lactams, >14 days since last dose	59	6 (10.2)	0.69 (0.27-1.76)	5 (8.5)	0.88 (0.33-2.35)
β-lactams, ≤14 days since last dose	53	3 (5.7)	0.28 (0.08-0.93)	2 (3.8)	0.34 (0.08-1.42)
L. gasseri present, *L. crispatus* and *L. jensenii* absent	2855	390 (13.7)		519 (18.2)	
None	2544	344 (13.5)	Referent	462 (18.2)	Referent
Antibiotics other than β-lactams	199	31 (15.6)	1.34 (0.86-2.09)	43 (21.6)	1.30 (0.85-2.00)
β-lactams, >14 days since last dose	59	13 (22.0)	1.52 (0.70-3.30)	11 (18.6)	0.81 (0.42-1.57)
β-lactams, ≤14 days since last dose	53	2 (3.8)	0.19 (0.04-0.82)	3 (5.7)	0.21 (0.05-0.98)

OR, odds ratio; CI, confidence interval

* β-lactams (penicillins, cephalosporins); all other antibiotics (macrolides, lincosamides, tetracyclines, fluoroquinolones, nitrofurantoin, sulfonamides, unknown).

6.0 MANUSCRIPT 3: ASSOCIATION OF CLASS-SPECIFIC ANTIBIOTIC USE, MULTPLE SEX PARTNERS, AND HORMONAL CONTRACEPTION WITH VUVOVAGINAL CANDIDIASIS

To be submitted for publication

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6.1 ABSTRACT

Objective: To evaluate risk factors for vulvovaginal candidiasis (VVC) in non-pregnant women.

Study Design: 650 asymptomatic women were followed bi-monthly for up to 18 months in a prospective cohort study. Behavioral information and antimicrobial and antifungal use were collected by interview. The risk of VVC, defined as clinician diagnosed yeast vaginitis or self-diagnosis with documented antifungal use, was evaluated using Andersen-Gill proportional hazards models for repeated events.

Results: The antibiotic use rate was 81/100 woman-years. There were 205 episodes of VVC over 860.8 woman-years for an incidence of 24/100 woman-years. After adjusting for age, number of sexual partners, and contraceptive method, women who used β-lactams (adjusted hazard ratio (aHR) =3.16, 95% confidence interval (CI): 2.06–4.86), metronidazole (aHR=2.52, 95% CI: 1.40–4.53), or fluoroquinolones and nitrofurantoin (aHR=2.31, 95% CI: 1.03–5.18) were more likely to develop VVC compared to women who did not use antibiotics. Other classes of antibiotics were not associated with VVC. Women using depot medroxyprogesterone acetate were 67% less likely to acquire VVC (aHR=0.33, 95% CI: 0.12–0.90) compared to women not using barrier or hormonal contraception. Other forms of hormonal contraception (tablets, vaginal rings, intrauterine devices, transdermal patches, and implants) were not associated with VVC. Compared to sexually abstinent women, women having multiple sex partners had an increased risk of VVC (aHR=4.59, 95% CI=1.87-11.28).

Conclusion: In this population of women having a high antibiotic use burden, the incidence of VVC varied by antibiotic class. Antibiotics, multiple sexual partners, and contraceptive choice influence the incidence of VVC.

6.2 INTRODUCTION

Vulvovaginal candidiasis (VVC) is characterized by vulval itching and abnormal vaginal discharge and is the second most common cause of vaginitis in reproductive aged women. It is estimated that as many as three out of four women will have VVC at least once in their lifetime.[33] The prevalence of yeast colonization is 20-25% among women of childbearing age with vaginal symptoms, while 10% of women are asymptomatically colonized by yeast in the vagina.[34, 35] Among women reporting an initial yeast infection, 10-25% reported 4 or more recurrent infections per year.[36] In addition to the discomfort and costs associated with VVC, some prospective studies have shown an increased risk of HIV acquisition among women who had VVC at the visit prior to seroconversion, independent of other risk factors. [38, 39]

While the pathogenesis of VVC remains unclear, several risk factors have been identified including systemic antimicrobial use, oral contraceptives, and vaginal *Lactobacillus* colonization.[33] However, the results of these studies have been inconsistent, particularly for antimicrobial use. A longitudinal study of 316 asymptomatic, non-pregnant women found no association between any antibiotic use and clinician-diagnosed or self-diagnosed yeast vaginitis.[46] A second case-control study of 691 women also reported no association between antibiotic use and culture-proven VVC.[45] By contrast, a prospective study of 80 women reported an association between any antibiotic use and increased incidence of VVC.[41] While a case-control study of 1,585 women also found an increased risk of VVC among women reporting any antibiotic use within the prior month, this association did not vary by antibiotic class.[43] The objective of this longitudinal study was to evaluate risk factors for VVC in non-pregnant women, with particular emphasis on class-specific antibiotic use and contraceptive methods.

6.3 METHODS

6.3.1 Study population

This is a secondary analysis of a randomized, clinical trial designed to evaluate the efficacy of a group B *Streptococcus* (GBS) serotype III polysaccharide-tetanus toxoid conjugated vaccine. Non-pregnant, sexually active women aged 18-40 years who were using effective birth control methods and were GBS culture-negative both vaginally and rectally were recruited from primary care and family planning settings in Pittsburgh, PA, Augusta, GA, and Houston, TX. Participants (n=663) were randomized to receive a single dose of GBS type III- tetanus toxoid vaccine or a licensed vaccine containing tetanus and diphtheria toxoids adsorbed for adult use. The clinical trial was approved by the institutional review boards of the participating institutions. All participants provided written informed consent. Enrollment occurred from July 2003 to August 2006, and all follow-up visits were completed by February 2008.

At the time of enrollment, women denied antimicrobial and antifungal use in the past 7 days and did not have VVC. Vaginal swabs for culture detection of lactobacilli, demographics, use of systemic or intra-vaginal antimicrobials and antifungals, contraceptive methods, and sexual activity were collected by pelvic examination and interview at enrollment, 1- and 2-months, and then bi-monthly for 18 months.

6.3.2 Vulvovaginal candidiasis and antibiotic use

VVC was defined as the report of a clinician-diagnosed yeast infection or self-diagnosed yeast infection with use of an antifungal documented by research personnel. Antimicrobial use was categorized by antibiotic class, and by mechanism of action as follows: β-lactams (penicillins, cephalosporins); protein synthesis inhibitors (macrolides, lincosamides, tetracyclines); nucleic acid synthesis inhibitors (fluoroquinolones, nitrofurantoin); and others (sulfonamides, class unknown). For all cases of VVC, participant records were reviewed to verify that the initiation of antimicrobial use preceded the onset of VVC. *Lactobacillus* were isolated and identified by methods that have been previously described.[76]

6.3.3 Covariates

The variables considered for analyses were study site, study arm, race, age, education, and marital and employment status at enrollment. Other variables which could change over time and for which data was collected at each visit included number of male sexual partners, new sex partner, frequency of vaginal intercourse, receptive anal intercourse, douching, Nugent score[77], vaginal *Lactobacillus* colonization, antibiotic use, and contraceptive methods. Contraceptives were categorized as none, non-barrier methods (spermicides, tubal ligations, or copper intrauterine devices), barrier methods (condoms, diaphragms, or cervical caps), hormonal methods (oral contraceptive tablets, intrauterine devices containing levonorgestrel, the vaginal ring, transdermal patches, or implants) and depot medroxyprogesterone acetate (DMPA).

6.3.4 Statistical analysis

Statistical analyses were performed using Stata statistical software release 11.2 (Stata Corp., College Station, TX), and statistical tests were evaluated at the 0.05 two-sided significance level. All women who completed at least one follow-up visit (N=650) were included in the analyses. Andersen-Gill proportional hazards models for repeated events were used to evaluate the factors associated with the incidence of VVC. Efron's method was used for handling tied failure times and the variance estimates were calculated using the method of Lin and Wei.[78, 94] Variables were considered for inclusion in multivariable models if the *P* value from the unadjusted model was less than 0.2. Variables were retained in the model if the *P* value from the Wald chi-squared test statistic was 0.05 or less. The final models were also adjusted for age. The proportional-hazards assumptions of the final models were tested on the basis of the scaled Schoenfeld residuals.[95]

6.4 RESULTS

This analysis included 650 women who completed 5599 follow-up visits over 860.8 woman-years. Most women (60%) completed all 10 scheduled follow-up visits and 91% completed 5 or more visits; the median follow-up time was 1.5 years. There were 205 episodes of VVC for an incidence of 24 per 100 woman-years (95% confidence interval (CI): 21-27). Of the 205 episodes, 164 were clinician diagnosed and 41 were self-diagnosed with documented use of an antifungal agent. There were no significant differences in the incidence of VVC between the three study sites or the two vaccine arms so the data were combined for further analyses. The incidence of VVC did not vary significantly by race, age, or years of education (Table 6.1).

Antimicrobial use was reported by 341 (52.5%) women at least once and at 605 (10.8%) of the follow-up visits. Women reported using a total of 699 antibiotic prescriptions for an overall antibiotic use rate of 81.2 per 100 woman-years of follow-up (95% CI: 75.4-87.5). Use of more than one antibiotic was reported at 75 visits; 2 at 52 visits, 3 at 21 visits, and 4 at 2 visits. The classes of antibiotics used are shown in Table 6.2. The most commonly reported antibiotics were β-lactams (30.8%), metronidazole (24.2%), and protein synthesis inhibitors (22.6%). The majority of antibiotics were used to treat genitourinary conditions (48.5%); primarily bacterial vaginosis (20.6%), urinary tract infections (13.3%), and sexually transmitted infections (10.9%). However, 26.2% of the antibiotic use was for the treatment of upper respiratory tract infections.

The unadjusted associations between antimicrobial use, contraceptive methods, sexual activity, and vaginal *Lactobacillus* colonization with VVC are shown in Table 6.3. Women who denied antibiotic exposure since the prior visit had an incidence of VVC of 20 per 100 woman-years (95% CI: 17-24), while women who reported any antibiotic use had an incidence of VVC of 52 per 100 woman-years (95% CI: 40-68, $p<0.001$). When antibiotic use was categorized by mechanism of action, the incidence of VVC ranged between 17 per 100 to 70 per 100 women-years with the highest incidence occurring among women who used β-lactams (penicillins, cephalosporins), followed by those who used metronidazole, and nucleic acid synthesis inhibitors (fluoroquinolones, nitrofurantoin, $p<0.001$). Among the 52 women who acquired VVC and had reported antibiotic use, the median duration of antibiotic therapy prior to the onset of symptoms was 5 days (range: 1-36 days), while the median time since first dose to onset of symptoms was 9 days (range: 1-63 days).

There was no significant difference in the incidence of VVC between women who did not use any or used non-barrier contraceptive methods (20 per 100 women-years), barrier methods

(25 per 100 women-years), and those who used oral contraceptives, intra-uterine devices containing levonorgestrel, the vaginal ring, transdermal patches, or implants (29 per 100 women-years). Compared to women using other contraceptive methods, women who used DMPA had a lower VVC incidence of 8 per 100 women-years (95% CI: 4-16, p=0.01), Table 6.3.

There was an association between various measures of sexual activity and increased incidence of VVC (Table 6.3). Women who were sexually abstinent since their prior visit had an incidence of 12 per 100 woman-years while the incidence was 24 per 100 women-years among women with one male partner, and 56 per 100 women-years among those with 2 or more partners (p=0.003). However, there was no significant dose-response relationship between frequency of sexual intercourse and VVC (data not shown). Finally, there was no association between vaginal *Lactobacillus* colonization at the prior visit, both any or hydrogen peroxide-producing strains, and VVC (p=0.12 for both).

In the multivariable regression analyses, antimicrobial use, DMPA, and numbers of male sex partners were independently associated with VVC (Table 6.4). When antibiotic use was included in the model as a dichotomous variable (Model I), women who used any antibiotic were 2.31 times more likely to acquire VVC (adjusted hazard ratio (aHR) =2.31, 95% CI: 1.67-3.19) compared to women who did not use antibiotics. When antibiotic use was categorized by mechanism of action (Model II), women who used β-lactams had a 3-fold increased risk of VVC (aHR =3.16, 95% CI: 2.06–4.86), while women who used metronidazole and nucleic acid synthesis inhibitors had a 2.4-fold increased risk (aHR=2.52, 95% CI: 1.40–4.53 and aHR=2.31, 95% CI: 1.03–5.18, respectively). Macrolides, lincosamides, tetracyclines and sulfonamides were not significantly associated with an increased risk of VVC. The adjusted hazard ratio for β-

lactams was significantly higher than that of sulfonamides and unknowns (p=0.047). However, the hazard ratios did not differ significantly among the other classes of antibiotics.

Compared with women who did not use any or used non-barrier contraceptive methods, women who used DMPA were 67% less likely to develop VVC (aHR=0.33, 95% CI: 0.12–0.90) (Model II). There was not a significant increased risk of VVC associated with oral contraceptives, intra-uterine devices containing levonorgestrel, the vaginal contraceptive ring, transdermal patches, or implants (aHR=1.07, 95% CI: 0.66–1.74) when compared to women that did not use any or used non-barrier contraceptives. The frequency of antibiotic use was similar between women who used DMPA (9.3%) and those who did not (10.7%, p=0.22).

The risk of VVC increased with number of male sex partners since the prior visit. Compared to women who were sexually abstinent since the last visit, women with one partner were 2 times more likely to acquire VVC (aHR=2.05, 95% CI: 1.03–4.08) and women with multiple partners were 4.6 times more likely to acquire VVC (aHR=4.59, 95% CI: 1.87–11.28), Model II. These analyses were repeated using only the 164 clinician-diagnosed VVC episodes as outcomes, and the results were similar (Table 6.5). In this analysis, the association between the use of fluoroquinolones and nitrofurantoin and VVC was no longer statistically significant (p=0.060). The proportional hazards assumption was not violated for any of the covariates included in the final models (p>0.16).

6.5 DISCUSSION

This study suggests that antimicrobial use was associated with a 2.3-fold increased risk of VVC which confirms those of previous studies.[41, 43] However, the magnitude of the association between antibiotic use and VVC varied by antibiotic class, with the highest risk being associated

with β-lactams, which was significantly different from the combined risk of VVC associated with sulfonamides. Metronidazole, fluoroquinolones, and nitrofurantoin were also associated with an increased risk of VVC acquisition, while other classes of antibiotics, including macrolides, lincosamides, tetracyclines, and sulfonamides were not associated with a significant increased risk of VVC. These results differ from those of Spinillo *et al* which did not report a difference in the risk of VVC among different antibiotic classes.[43] The present study prospectively evaluated antibiotic use and its association with incident clinician- or self-diagnosed VVC whereas the Spinillo *et al* study was retrospective in nature and evaluated prevalent culture-proven VVC. The contradicting results of the two studies may also be due to regional differences in antibiotic prescribing patterns. The antibiotic use rate among the women in this study was 81.2 per 100 person-years, which is considerably higher than the estimated national outpatient prescribing rate of 30 per 100 among adults in 2002.[50] While the burden of antibiotic use in this population of reproductive-aged women was high, a quarter of the antibiotic use was for upper respiratory tract infections for which antibiotics are rarely recommended.[79] Since the results from the current study indicate that antibiotics increase the risk for acquiring VVC, clinicians should refrain from prescribing unnecessary antibiotics in women.

Although many consumer-oriented web sites and printed materials cite the dogma that oral contraceptives and other hormonal methods increase yeast vaginitis, in this population of sexually active women, VVC was not increased among women using hormonal contraceptive methods. Women using DMPA had a decreased risk of VVC, a finding consistent with those of Miller *et al* who reported a decrease in vaginal yeast colonization from 21% at baseline to 8% at 3 and 6 months after initiation of DMPA use (P=0.02).[96] However, our results differ from those of Baeten *et al* who reported no decrease in incident vaginal candidiasis among 251 African

women using DMPA.[97] Since VVC is an estrogen-dependent condition,[98] it is biologically plausible that DMPA would reduce VVC due to the hypoestrogenic effect of this contraceptive method. DMPA induces a profound suppression of estradiol levels via negative feedback on the hypothalamic-pituitary-ovarian axis, resulting in estradiol levels which may become as low as those observed in post-menopausal women.[99] This concept is supported by animal model studies which have demonstrated that the presence of estrogen decreased the capacity of vaginal epithelial cells to inhibit the growth of *Candida albicans* in mice.[100] To help determine whether a similar situation occurs in humans, future studies should evaluate the association between the incidence of VVC and contraceptive methods containing only progesterone and those also containing estrogen .

Although oral contraceptives were not associated with VVC in our study, Baeten *et al* reported an increased risk of vaginal candidiasis among sex workers in Africa using oral contraceptives. [97] However, differences in the study population and their sexual activity may account for this inconsistency since the risk of VVC increased with numbers of male sex partners in the current study.

Sexual activity, as measured by number of male sexual partners, was associated with VVC in our study, and there was a dose-response relationship with increasing numbers of partners. In contrast, there was no dose-response relationship with frequency of sexual intercourse. While previous studies have shown an association between various sexual behaviors and increased VVC, they did not find a significant association with numbers of sex partners.[101-103] The mechanisms by which increased numbers of sex partners increase risk of VVC deserves further study but it is plausible that sexual activity increases transfer of yeast from the mouth or

rectum to the vagina, and that having more partners increased the likelihood of exposure to a male partner colonized by yeast at these sites.

Because women vaginally colonized by lactobacilli have a decreased incidence of bacterial vaginosis, HIV, and other sexually transmitted pathogens,[104-106] many have presumed that lactobacilli should also decrease the risk of VVC. Paradoxically, one study found that women with concurrent vaginal *Lactobacillus* colonization had a 4-fold increased risk of VVC.[107] In contrast, there was no association between colonization by vaginal lactobacilli at the prior visit and VVC in this study which suggest that *Lactobacillus* colonization is neither protective nor a risk factor for VVC. Our results are consistent with those of Hawes *et al* who found that the presence of hydrogen peroxide-producing lactobacilli did not protect against acquisition of VVC in 182 women.[105] While the effect of antibiotic use on colonization by lactobacilli was not assessed, penicillins and cephalopsorins which have activity against lactobacilli and metronidazole which does not, all were associated with an increased risk of yeast vaginitis so it is unlikely that the effect of antimicrobial use on vaginal lactobacilli colonization is important in the pathogenesis of VVC.

Major strengths of our study include the prospective longitudinal design and the large sample of 650 women with over 5500 visits. However, there are also limitations. VVC episodes were identified through participant reports; therefore the diagnosis of yeast vaginitis could not be confirmed. In addition, 20% of the VVC events were self-diagnosed. However, the associations of antibiotic use, contraceptive methods, and sexual activity with VVC remained unchanged when the analyses were restricted to clinician diagnosed VVC events. Antibiotic exposure in the last 2 months was ascertained by interview which is subject to recall error yet a recent study found 89% agreement between self-reported antibiotic use in the last 3 months and pharmacy

database records [87] Finally, this was a young, sexually active population which limits generalizability of results; results may not be applicable to adolescents, pregnant women, or older women.

This study suggests that the association of antibiotic use with increased VVC varies by antibiotic classes which may explain the inconsistencies of previous studies which have evaluated antibiotic use as a dichotomous variable. The use of DMPA had a protective effect while other hormonal contraceptive methods, such as oral contraceptives, were not associated with VVC. While the etiology of VVC is complex, antibiotic therapy, number of male sex partners, and contraceptive choices influence the incidence of VVC.

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6.7 TABLES

Table 6.1 Association of enrollment characteristics with vulvovaginal candidiasis

	No. of participants	No. of VVC events	Rate* of VVC (95% CI)	Unadjusted HR (95% CI)	*P*-value
Site					
Pittsburgh, PA	391	147	25 (21-29)	Referent	
Augusta, GA	212	50	21 (16-28)	0.86 (0.59-1.26)	0.44
Houston, TX	47	8	24 (12-49)	0.97 (0.35-2.69)	0.95
Vaccine arm					
Tetanus-diphtheria	330	114	26 (22-31)	Referent	
GBS type III	320	91	22 (18-26)	0.82 (0.58-1.16)	0.27
Race					
White	391	122	23 (20-28)	Referent	
Black	212	71	25 (20-32)	1.13 (0.77-1.66)	0.52
Other	47	12	21 (18-37)	0.91 (0.47-1.76)	0.77
Age, years					
18-19	67	15	17 (10-28)	Referent	
20-24	274	103	29 (24-35)	1.69 (0.92-3.13)	0.09
25-29	167	53	24 (18-31)	1.39 (0.70-2.76)	0.34
30-34	72	21	22 (15-34)	1.33 (0.60-2.94)	0.49
35-40	70	13	13 (8-23)	0.81 (0.33-1.97)	0.64
Education, years					
8-12	162	41	19 (14-26)	Referent	
13-16	347	119	26 (22-32)	1.35 (0.86-2.10)	0.19
17 or more	141	45	23 (17-31)	1.17 (0.69-1.97)	0.56

VVC, vulvovaginal candidiasis; HR, hazard ratio; CI, confidence interval

* Rate per 100 person-years

Table 6.2 Antibiotic use by class

Antibiotic Class	No. of Prescriptions Reported	Antibiotic Use Rate* (95% CI)
Total prescriptions	699	81.2 (75.4-87.5)
β-lactams	215 (30.8%)	25.0 (21.9-28.5)
Penicillins	158 (22.6%)	18.4 (15.7-21.5)
Cephalosporins	57 (8.2%)	6.6 (5.1-8.6)
Metronidazole	169 (24.2%)	19.6 (16.9-22.8)
Protein synthesis inhibitors	158 (22.6%)	18.4 (15.7-21.5)
Macrolides	106 (15.2%)	12.3 (10.2-14.9)
Tetracyclines	42 (6.0%)	4.9 (3.6-6.6)
Lincosamides	10 (1.4%)	1.2 (0.6-2.2)
Nucleic acid synthesis inhibitors	79 (11.3%)	9.2 (7.4-11.4)
Fluoroquinolones	61 (8.7%)	7.1 (5.5-9.1)
Nitrofurantoin	18 (2.6%)	2.1 (1.3-3.3)
Others	78 (11.2%)	9.1 (7.3-11.3)
Sulfonamides	47 (6.7%)	5.5 (4.1-7.3)
Aminoglycosides	1 (0.1%)	0.1 (0.02-0.8)
Unknown	30 (4.3%)	3.5 (2.4-5.0)

CI, confidence interval

* Number of antibiotic uses per 100 person-years

Table 6.3 Association of antibiotic use, contraceptive methods, sexual activity, and vaginal *Lactobacillus* colonization with vulvovaginal candidiasis

	No. of VVC events	Rate* of VVC (95% CI)	Unadjusted HR (95% CI)	*P*-value
Antibiotic use since last visit				
Any	52	52 (40-68)	2.47 (1.78-3.43)	<0.001
None	153	20 (17-24)	Referent	
Antibiotic use since last visit†				
None	153	20 (17-24)	Referent	
β-lactams	25	70 (47-104)	3.20 (2.06-4.95)	<0.001
Protein synthesis inhibitors	8	37 (19-75)	1.85 (0.87-3.90)	0.11
Metronidazole	11	55 (30-99)	2.74 (1.54-4.91)	0.001
Nucleic acid synthesis inhibitors	6	54 (24-119)	2.50 (1.11-5.63)	0.03
Others	2	17 (4-70)	0.81 (0.20-3.28)	0.77
Contraception since last visit‡				
None or non-barrier methods	34	20 (14-27)	Referent	
Barrier methods	45	25 (19-33)	1.27 (0.78-2.09)	0.34
Hormonal methods, not DMPA	119	29 (24-34)	1.43 (0.91-2.25)	0.12
DMPA	7	8 (4-16)	0.39 (0.15-1.06)	0.06
New sex partner since last visit				
Yes	27	43 (29-63)	1.91 (1.28-2.85)	0.002
No	178	22 (19-26)	Referent	
Number of sex partners since last visit				
None	12	12 (7-20)	Referent	
One	174	24 (21-28)	2.10 (1.07-4.13)	0.03
Two or more	19	56 (36-88)	4.84 (1.95-11.98)	0.001
Vaginal *Lactobacillus* colonization at prior visit				
Present	182	25 (22-29)	1.46 (0.91-2.33)	0.12
Absent	23	17 (12-26)	Referent	
Vaginal H_20_2-producing *Lactobacillus* colonization at prior visit				
Present	156	26 (22-30)	1.35 (0.92-1.97)	0.12
Absent	49	19 (15-26)	Referent	

VVC, vulvovaginal candidiasis; HR, hazard ratio; CI, confidence interval; DMPA, depot medroxyprogesterone acetate

* Rate per 100 person-years

† β-lactams (penicillins, cephalosporins); protein synthesis inhibitors (macrolides, lincosamides, tetracyclines); nucleic acid synthesis inhibitors (fluoroquinolones, nitrofurantoin); others (sulfonamides, unknown).

‡ Non-barrier methods (spermicides, tubal ligations, copper intrauterine devices); barrier methods (condoms, diaphragms, cervical caps); hormonal methods, not DMPA (oral contraceptive tablets, vaginal rings, intrauterine devices containing levonorgestrel, transdermal patches, implants).

Table 6.4 Independent risk factors for vulvovaginal candidiasis, both clinician and self-diagnosed events (N=5599 Visits, n=205 events)

	Adjusted* Hazards Ratio	95% Confidence Interval	*P*-value
Model I			
Any antibiotic use since last visit	2.31	1.67 – 3.17	<0.001
Contraception since last visit‡			
None or non-barrier methods	1.00	referent	
Barrier methods	0.98	0.58 – 1.66	0.94
Hormonal methods, not DMPA	1.07	0.65 – 1.74	0.80
DMPA	0.33	0.12 – 0.90	0.031
Number of sexual partners since last visit			
None	1.00	referent	
One	2.02	1.02 – 4.04	0.045
Two or more	4.45	1.80 – 11.00	0.001
Model II			
Antibiotic use since last visit†			
None	1.00	referent	
β-lactams	3.16	2.06 – 4.86	<0.001
Protein synthesis inhibitors	1.63	0.78 – 3.40	0.19
Metronidazole	2.52	1.40 – 4.53	0.002
Nucleic acid synthesis inhibitors	2.31	1.03 – 5.18	0.042
Others	0.72	0.18 – 2.97	0.65
Contraception since last visit‡			
None or non-barrier methods	1.00	referent	
Barrier methods	0.99	0.59 – 1.66	0.98
Hormonal methods, not DMPA	1.07	0.66 – 1.74	0.77
DMPA	0.33	0.12 – 0.90	0.030
Number of sexual partners since last visit			
None	1.00	referent	
One	2.05	1.03– 4.08	0.041
Two or more	4.59	1.87 – 11.28	0.001

DMPA, depot medroxyprogesterone acetate

***** Adjusted hazards ratio obtained with Andersen-Gill proportional hazards regression model including age, antimicrobial use, contraceptive method, and number of sexual partners.

† β-lactams (penicillins, cephalosporins); protein synthesis inhibitors (macrolides, lincosamides, tetracyclines); nucleic acid synthesis inhibitors (fluoroquinolones, nitrofurantoin); others (sulfonamides, unknown).

‡ Non-barrier methods (spermicides, tubal ligations, copper intrauterine devices); barrier methods (condoms, diaphragms, cervical caps); hormonal methods, not DMPA (oral contraceptive tablets, vaginal rings, intrauterine devices containing levonorgestrel, transdermal patches, implants).

Table 6.5 Independent risk factors for vulvovaginal candidiasis, restricted to clinician-diagnosed events (N=5599 Visits, n=164 events)

	Adjusted* Hazards Ratio	95% Confidence Interval	*P*-value
Model I			
Any antibiotic use since last visit	2.32	1.62 – 3.31	<0.001
Contraception since last visit‡			
None or non-barrier methods	1.00	referent	
Barrier methods	0.74	0.42 – 1.30	0.30
Hormonal methods, not DMPA	0.97	0.58 – 1.61	0.90
DMPA	0.26	0.08 – 0.78	0.017
Number of sexual partners since last visit			
None	1.00	Referent	
One	2.36	1.16 – 4.81	0.018
Two or more	3.66	1.39 – 9.65	0.009
Model II			
Antibiotic use since last visit†			
None	1.00	referent	
β-lactams	3.29	2.09 – 5.18	<0.001
Protein synthesis inhibitors	1.55	0.63 – 3.80	0.34
Metronidazole	2.59	1.34 – 5.00	0.005
Nucleic acid synthesis inhibitors	2.38	0.96 – 5.90	0.060
Others	0.46	0.06 – 3.32	0.44
Contraception since last visit‡			
None or non-barrier methods	1.00	Referent	
Barrier methods	0.76	0.43 – 1.32	0.32
Hormonal methods, not DMPA	0.97	0.59 – 1.62	0.92
DMPA	0.26	0.08 – 0.76	0.016
Number of sexual partners since last visit			
None	1.00	Referent	
One	2.39	1.17 – 4.86	0.016
Two or more	3.81	1.46 – 9.95	0.006

DMPA, depot medroxyprogesterone acetate

* Adjusted hazards ratio obtained with Andersen-Gill proportional hazards regression model including age, antimicrobial use, contraceptive method, and number of sexual partners.

† β-lactams (penicillins, cephalosporins); protein synthesis inhibitors (macrolides, lincosamides, tetracyclines); nucleic acid synthesis inhibitors (fluoroquinolones, nitrofurantoin); others (sulfonamides, unknown).

‡ Non-barrier methods (spermicides, tubal ligations, copper intrauterine devices); barrier methods (condoms, diaphragms, cervical caps); hormonal methods, not DMPA (oral contraceptive tablets, vaginal rings, intrauterine devices containing levonorgestrel, transdermal patches, implants).

7.0 DISCUSSION

7.1 SUMMARY OF FINDINGS

The objectives of this research were to describe the burden of antibiotic use in young women, to assess the impact of antibiotics on vaginal and rectal lactobacilli, and to evaluate the association between antibiotic use and incident yeast vaginitis. Antibiotic use was widespread in this population of relatively healthy, reproductive-aged, non-pregnant women. The majority of the antibiotic use was for the treatment of genitourinary infections. Factors associated with use of antibiotics for indications other than upper respiratory tract infections included having more sexual partners, black race, and the absence of predominant vaginal lactobacilli, which are known risk factors for genital tract infections. However, about one in five antibiotics prescribed were used to treat upper respiratory tract illnesses for which antibiotics provide little therapeutic benefit. The treatment of upper respiratory tract infections was the major contributor to exposure of women to broad spectrum and β-lactam antibiotics in this study. Factors associated with possible inappropriate antibiotic use were being white and older age which may be markers for socio-economic factors associated with differential access to care. Paradoxically, although black women in this study were more likely to have received antibiotics, they were less likely to have been prescribed antibiotics inappropriately for upper respiratory tract infections.

A major concern related to the inappropriate use of antibiotics is that this use will result in the selection of antibiotic resistant microbes. The present study did not evaluate the impact of antibiotic use on the antibiotic susceptibility of pathogens, but rather focused on the impact of antibiotic use on lactobacilli, which are the predominant members of the healthy vaginal

microbiome. Because lactobacilli are gram positive bacteria having susceptibility to several antibiotic classes, it is biologically plausible that antibiotic use could have a profound impact on colonization by this beneficial microbe. However, this study showed that many antibiotics had no measurable effect on colonization by lactobacilli, and the effect was limited to the use of β-lactam agents. For this study, we focused on the species of lactobacilli which produce hydrogen peroxide because it has been shown that colonization by hydrogen peroxide-producing lactobacilli is associated with a reduced risk of acquiring bacterial vaginosis. There was a significant reduction in vaginal and rectal colonization by hydrogen peroxide-producing lactobacilli following the use of β-lactam antibiotics, while other classes of antibiotics or overall self-reported antibiotic use were not associated with decreased *Lactobacillus* colonization in our population of women. The class-specific effect of β-lactam antibiotics likely accounts for the inconsistencies in the published literature which have reported on the impact of antibiotic use on vaginal lactobacilli.

All of the published studies which have evaluated the impact of antibiotic use on colonization by lactobacilli have focused on the vagina, since this microorganism is the predominant microorganism present in this ecosystem. A key strength of this research was the inclusion of rectal swabs which allowed for the simultaneous detection of lactobacilli in both body sites at the same sampling interval. A novel finding from the present study was that use of β-lactams reduced rectal colonization by hydrogen peroxide-producing lactobacilli to a similar degree as that observed in the vagina. However, the impact of β-lactam use on rectal colonization persisted for longer than that observed for the vagina. Only β-lactam use in the previous two weeks was associated with reduced vaginal colonization. These results suggest that the disruption of *Lactobacillus* colonization associated with the use of β-lactam antibiotics persists

in the rectum for a longer period of time than in the vagina, which is consistent with the concept that there is greater perturbation of the gut microflora following antibiotic use than the microflora at other anatomical sites.

There are several different species of lactobacilli present in the vaginal and rectal microbiome. In this population of reproductive-aged women, the use of β-lactam antibiotics was associated with reduced colonization by any hydrogen peroxide-producing lactobacilli. Another novel contribution of the present study was the identification of the individual species present in the vagina and rectum in order to assess whether the impact of antibiotic usage on colonization was species- or genus-specific. There was variability in the inhibitory effect of β-lactam antibiotics on the primary hydrogen peroxide-producing species, *L. crispatus*, *L.jensenii*, and *L. gasseri*. While the use of β-lactams was associated with decreased colonization by *L. crispatus* in the rectum but not in the vagina, recent β-lactam use was associated with marked decreases in both vaginal and rectal *Lactobacillus* colonization in women whose predominant hydrogen peroxide-producing *Lactobacillus* species were *L. jensenii* or *L. gasseri*.

The final set of analyses evaluated whether the high burden of antibiotic use and disruption of the vaginal microbiota was associated with increased numbers of symptomatic yeast infections. Self-reported antibiotic use was associated more than a 2-fold increased risk of incident vulvovaginal candidiasis in this cohort of non-pregnant, reproductive-aged women. However, the magnitude of the association between antibiotic use and vulvovaginal candidiasis varied by antibiotic class, with the highest risk being associated with β-lactams, followed by metronidazole, and nucleic acid synthesis inhibitors (fluoroquinolones and nitrofurantoin). Other classes of antibiotics, including macrolides, lincosamides, tetracyclines, and sulfonamides were not associated with a significant increased risk of vulvovaginal candidiasis which may explain

the inconsistencies of previous studies which have evaluated antibiotic use as a dichotomous variable.

In summary, the analyses conducted as part of this dissertation have confirmed that antibiotic use is common in young women and extended our knowledge by showing that the preponderance of antibiotic usage is for the treatment of genitourinary tract infections. However, the present study did find an increased prevalence of inappropriate use of antibiotics for upper respiratory tract infections among older and white women. These data suggest that programs to reduce antibiotic use will require multiple strategies comprising of reduction in treatable genitourinary tract infections and reducing the use of antibiotics for upper respiratory tract infections. Our data also suggest that the use of β-lactam antibiotics deserves special attention in the efforts to reduce inappropriate use of antibiotics since this class of antibiotics was associated with decreased colonization by beneficial lactobacilli and the greatest increase in incident yeast vaginitis. Together, these analyses have provided new insights into the usage of antibiotics and their broad impact on the health of reproductive-aged women.

7.2 FUTURE RESEARCH

The research presented here utilized data obtained as part of a randomized clinical trial, and thus the study was not optimally designed to evaluate the impact of antibiotic use on *Lactobacillus* colonization and incident yeast vaginitis. Future prospective studies could expand upon the current research by enrolling women who are prescribed an antibiotic for any indication at an office or clinic visit. Vaginal and rectal samples for microbiological cultures could then be obtained prior to the initiation of antibiotic therapy, followed by the collection of daily samples for the first 2-3 weeks, and then weekly samples for two months. This sampling schedule would

enable evaluation of the vaginal and rectal microflora prior to initiation and after cessation of antibiotic therapy in order to assess the acute effects of specific antibiotics on each microbiome and also estimate the length of the impact of antibiotic use. In addition, this type of study design would allow for proactive clinical assessments of vaginal infections, such as yeast vaginitis and bacterial vaginosis, which may lead to a better understanding of the mechanisms by which antibiotics may enhance the development of these conditions.

7.3 PUBLIC HEALTH SIGNIFICANCE

Minimizing the inappropriate use of antibiotics is a public health priority. Since studies indicate a high prevalence of over-prescribing antibiotics for the treatment of upper respiratory tract infections, many antibiotic stewardship programs focus on reducing inappropriate antibiotic use for the treatment of these conditions. Reducing unnecessary antibiotic treatment of upper respiratory tract infections would reduce exposure to broad-spectrum antibiotics and to β-lactams since they were primarily used to treat these illnesses in our population of young women. This would have a beneficial impact on public health for several reasons. The use of β-lactams was the only class of antibiotics associated with decreased colonization by hydrogen peroxide-producing lactobacilli. Women lacking hydrogen-producing lactobacilli in the vagina and/or rectum have an increased incidence of bacterial vaginosis, which subsequently increases the risk of acquiring other genital infections such as HIV, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Trichomonas vaginalis.* In our population of women, β-lactams were also associated with the highest risk of acquiring yeast vaginitis, the second most common cause of vaginitis after bacterial vaginosis. Finally, the use of broad-spectrum antibiotics contributes to the development of antibiotic-resistant infections. Therefore, it would be important to tailor

programs promoting the judicious use of antibiotics for the treatment of upper respiratory tract infections to women and their healthcare providers to increase awareness that the inappropriate use of antibiotics may disrupt the normal vaginal and rectal microbiota and increase susceptibility to other infections. However, targeting only the treatment of upper respiratory tract infections would have little impact on the overall antibiotic use in reproductive-aged women since the majority of the antibiotic use was for the treatment of genitourinary conditions. Prevention efforts on reducing acquisition of sexually transmitted infections would have the cross benefit of also reducing antibiotic exposure in women.

Yeast vaginitis accounts for millions of office visits each year for reproductive aged women. Overall antibiotic use was associated with yeast vaginitis which confirms many patient and clinician perceptions. However the risk associated with increased incidence of vulvovaginal candidiasis varied by antibiotic class, even after accounting for other risk factors (sexual activity and contraception). The pathophysiology of vulvovaginal candidiasis is complex and poorly understood even after decades of research. The classes of antibiotics that were associated with vulvovaginal candidiasis had different mechanisms of action which suggests that they may be affecting a different component in the physiological pathway. It is likely that antibiotic use creates a temporary imbalance in the vaginal microbiome, allowing for proliferation of indigenous yeasts. Sexual activity was also associated with vulvovaginal candidiasis, which not only creates an imbalance in the vaginal ecosystem, but allows for transfer of yeasts from the rectum or from a colonized sexual partner to the vagina. These findings suggest that women with a history of recurrent yeast vaginitis should be advised that antibiotic use may increase their risk of vulvovaginal infections.

The introduction of antibiotics in clinical care led to a significant reduction in mortality due to infectious diseases in the US in the early 1900s.[1] However, the overuse of antibiotics has since contributed to the emergence of antibiotic resistant bacteria which are estimated to cause more than 2 million illnesses and 23,000 deaths per year.[3] While the primary objective in reducing unnecessary exposure to antibiotics is to curb the emergence and spread of antibiotic resistant pathogens, reducing exposure to antibiotics, particularly to β-lactams which is the most common class of antibiotics prescribed in the US, will have the additional benefit of minimizing the adverse effects of antibiotic use on the beneficial indigenous vaginal and rectal microflora of reproductive-aged women.

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